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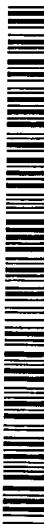
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(54) Title: NON-COVALENT INHIBITORS OF UROKINASE AND BLOOD VESSEL FORMATION

(57) Abstract: Novel compounds having activity as non-covalent inhibitors of urokinase and having activity in reducing or inhibiting blood vessel formation are provided. These compounds have P1 a group having an amidino or guanidino moiety or derivative thereof. These compounds are useful in vitro for monitoring plasminogen activator levels and in vivo in treatment of conditions which are ameliorated by inhibition of or decreased activity of urokinase and in treating pathologic conditions wherein blood vessel formation is related to a pathologic condition.

DESCRIPTIONNon-Covalent Inhibitors of Urokinase and
Blood Vessel Formation5 Cross Reference to Related Applications

This application is a continuation-in-part of United States Serial No. 09/733,645, filed December 7, 2000, which is a continuation-in-part of United States Serial No. 09/637,483, filed August 11, 2000, the disclosures of which 10 is incorporated by reference herein.

Field of the Invention

Urokinase is an enzyme involved in the metastasis of tumor cells, neovascularization, and other activities. One purpose of the present invention is to provide novel 15 compounds which are active as inhibitors of urokinase that can be used to inhibit the activity of urokinase and thereby attenuate its deleterious effects. Another purpose of the present invention is to provide novel compounds which inhibit blood vessel formation, particularly blood vessel 20 formation related to a pathologic condition.

Background and Introduction to the Invention

Urinary-type plasminogen activator (uPA; urokinase) is a serine protease within the trypsin/chymotrypsin family. In its physiological state, uPA is found in three forms: 25 single chain pro-uPA, two chain uPA, and low molecular weight uPA (lacks N-terminal domains). The zymogen, pro-uPA, is converted to u-PA by cleavage of the peptide bond at K158-I159. The resultant two chain uPA is linked by

disulfide bridges, has an M_r of about 50 kD, and a C-terminal serine proteinase domain.

The activity of uPA is focused to cell surfaces upon binding to its receptor, uPAR. uPAR is a single-chain glycosyl phosphatidyl inositol (GPI)-anchored membrane receptor. The N-terminal 92 amino acids of uPAR play a dominant role in binding to uPA and pro-uPA. Receptor for uPA has been located on T-cells, NK cells, monocytes, and neutrophils, as well as vascular endothelial cells, fibroblasts, smooth muscle cells, keratinocytes, placental trophoblasts, hepatocytes, and a wide variety of tumor cells.

After conversion of pro-uPA to uPA, which occurs primarily at the uPAR on the cell surface, uPA activates plasminogen to plasmin. Activation occurs upon cleavage at residues PGR-VV for human plasminogen, or at residues SGR-IV for bovine plasminogen. Because plasminogen also is present on the cell surface, this activation cascade focuses the activity of u-PA and plasmin on the plasma membrane..

Plasmin has many roles, including activation of additional uPA and other enzymes, digestion of fibrin, and digestion of components of the extracellular matrix (ECM). Digestion of the ECM surrounding a tumor removes the ECM as a physical barrier to metastasizing cells, which are then free to leave primary tumors and invade secondary sites. A review of the role of the uPA/uPAR system in cancer metastasis is provided in "The Urokinase-type Plasminogen Activator System in Cancer Metastasis: A Review", Andreassen *et al.*, Int. J. Canc. 72:1-22 (1997).

A correlation between a high level of uPA and a high rate of metastasis, and poor prognosis, has been noted in certain tumors, especially breast cancer [Quax *et al.*, J.

Cell Biol. 115:191-199 (1991); Duffy et al., Cancer Res. 50:6827-6829 (1990)]. For instance, tumors of the lung [Oka et al., Cancer Res. 51:3522-3525 (1991)], bladder [Hasui et al., Int. J. Cancer 50:871-873 (1992)], stomach [Nekarda et al., Lancet 343:117 (1994)], cervical cancer [Kobayashi et al., Cancer Res. 54:6539-6548 (1994)], ovary [Kuhn et al., Gynecol. Oncol. 55:401-409 (1994)], kidney [Hofmann et al., Cancer 78:487-492 (1996)], brain [Bindahl et al., J. Neuro-Oncol. 22:101-110 (1994)], and soft tissue sarcoma [Choong et al., Int. J. Cancer (Pred. Oncol.) 69:268-272 (1996)] have exhibited a high level of uPA and/or uPA activity and a high rate of metastases. Overproduction of uPA has been reported to result in increased skeletal metastasis by prostate cancer cells *in vivo* [Achbarou et al., Cancer Res. 54:2372-2377 (1994)].

Inhibition or lowering of uPA activity, or disruption/inhibition of the interaction between uPA and its receptor (uPAR) has been shown to have a positive effect on maintenance of the extracellular matrix and an inhibitory effect on metastasis [Ossowski and Reich, Cell 35:611-619 (1983); Ossowski, Cell 52:321-328 (1988); Ossowski, J. Cell Biol. 107:2437-2445 (1988); Wilhelm et al., Clin. Exp. Metastasis 13:296-302 (1995); Achbarou et al., Cancer Res. 54:2372-2377 (1994); Crowley et al., Proc. Natl. Acad. Sci. USA 90:5021-5025 (1993); Kook et al., EMBO J. 13:3983-3991 (1994)]. The results of such experimental studies suggest that uPA-catalyzed plasminogen activation is rate-limiting for tumor progression, local tumor invasion and/or formation of distant metastasis. [Andreasen et al., Int. J. Canc. 72:1-22 (1997)].

The effects of the uPA system on cell migration and invasion are thought to be due to both a proteolytic effect

of plasmin-mediated degradation of the extracellular matrix, as well as more a direct interaction of the uPA receptor with components of the extracellular matrix. Degradation of the extracellular matrix permits a metastasizing cell to 5 invade the matrix, whereas interaction between uPA receptor and the matrix itself assists a cell in its migration. Localization of the uPA/plasmin system on the cell surface, or the leading edge of metastasizing cells, is consistent with postulated role of uPA in metastasis [Plesner et al., 10 Stem Cells 15:398-408 (1997)].

Interaction of uPAR with vitronectin, a component of the extracellular matrix, mediates cell adhesion and can be enhanced when uPAR is bound by uPA. Cell surface adhesion molecules, integrins, also appear to be involved in this 15 adhesion function, particularly beta-1 and beta-2 integrins [Paysant et al., Br. J. Haematol. 100:45-51 (1998); Simon et al., Blood 88:3185-3194 (1996)]. The CD11b/CD18 integrin can associate with the uPA-uPAR complex and promote adhesion of cells bearing these receptors, e.g., neutrophils, 20 leukocytes.

The uPA/uPAR system also is involved in the establishment of new vasculature, or neovascularization.

Establishment of new vasculature is required for sustaining primary and metastatic tumor growth.

25 Pathological neovascularization also is a characteristic of retinal disease, rubeosis iritis, proliferative vitreoretinopathy inflammatory disease, diabetic retinopathy, chronic uveitis, Fuch's heterochromic iridocyclitis, neovascular glaucoma, corneal or optic nerve 30 neovascularization, vascular disease, pterygium, glaucoma surgery bleb failure, hyperkeratosis, cheloid and polyp formation (see EP 451,130). Undesired angiogenesis also can

occur in the following conditions or can be a result of the following activities: macular degeneration, retinopathy of prematurity, corneal graft rejection, retrothalental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sogrens disease, acne rosacea, phylectenulosis, syphilis, Mycobacteria infections other than leprosy, lipid degeneration, chemical burns, bacterial or fungal ulcers,

5 Herpes simplex or zoster infections, protozoan infections, Kaposi's sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegeners sarcoidosis, scleritis, Steven's Johnson disease, radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein or artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, myopia, optic pits, Stargarts disease, pars planitis, chronic

10 retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular tissue, hemangiomas, Osler-Weber-Rendu, solid tumors, blood borne tumors, AIDS, ocular neovascular disease, osteoarthritis, chronic inflammation, Crohn's

15 disease, ulcerative colitis, tumors of rhabdomyosarcoma, tumors of retinoblastoma, tumors of Ewing sarcoma, tumors of neuroblastoma, tumors of osteosarcoma, leukemia, psoriasis, atherosclerosis, pemphigoid, as recited in U.S. Patent No. 5,712,291.

20 An antagonist of uPA/uPAR binding (EGF-like domain of uPA fused to Fc of IgG) was said to inhibit neovascularization and growth of the murine B16 melanoma.

[Min et al., *Cancer Res.* 56:2428-2433 (1996)]. Consistent with this finding is the correlation noted between microvessel density, vascular invasion and uPA levels in breast carcinomas [Hildenbrand et al., *Brit. J. Cancer* 72:818-823 (1995)]. The known uPA inhibitor amiloride also was said to inhibit a variety of neovascularization pathologies [Glaser et al., EP 451,130; Avery et al., *Arch. Ophthalmol.* 108:1474-1476 (1990)].

There are two primary physiological inhibitors of uPA, PAI-1 and PAI-2, which are members of the serpin family of proteinase inhibitors. The binding of serpins to their cognate proteases involves a large number of interactions between amino acids of each protein, including those in the serpin reactive loop (Ser-Ala-Arg-Met-Ala (SEQ. ID. NO. 1) for PAI-1, Thr-Gly-Arg-Thr-Gly (SEQ. ID. NO. 2) for PAI-2). Introduction of exogenous PAI-2 into experimental animals was reported to inhibit the rate of lung metastasis [Evans and Lin, *Amer. Surg.* 61:692-697 (1995); Mueller et al., *Proc. Natl. Acad. Sci. USA* 92:205-209 (1995)]. The ability of PAI-1 to inhibit metastasis has not yet been consistently shown. The gene for PAI-1, and means for its recombinant expression, are disclosed in Loskutoff et al., U.S. Patent No. 4,952,512. Recombinant and native human PAI-2 is disclosed in Stephens et al., U.S. Patent No. 5,422,090.

The most widely studied uPA inhibitors may be within the 4-substituted benzo[b]thiophene-2-carboxamidine class of inhibitors, of which B428 (4-iodo-benzo[b]thiophene-2-carboxamidine) and B623 are members [Towle et al., *Cancer Res.* 53:2553-2559 (1993); Bridges et al., *Bioorg. Med. Chem.* 1:403-410 (1993); Bridges et al., U.S. Patent No. 5,340,833]. Infusion of B428 in experimental rats inoculated with tumor cells was said to inhibit uPAR gene

expression, decrease the primary tumor volume and decrease metastases [Xing et al., *Cancer Res.* 57:3585-3593 (1997)]. Daily intraperitoneal treatment of mice bearing tumors with B428 or B623 was said to block metastasis to muscle and fat, 5 but did not inhibit tumor-induced angiogenesis or reduce the rate of spontaneous lung metastasis. In fact, B623 enhanced the formation of lung metastasis (Alonso et al., *Breast Cancer Res. Treat.* 40:209-223 (1996)). Infusion of B428 in a syngeneic model of rat prostate cancer also lead to a 10 decrease in primary tumor volume and tumor weight, and a decrease in metastasis [Rabbani et al., *Int. J. Cancer* 63:840-845 (1995)].

Other known inhibitors of uPA include p-aminobenzamidine, which is a competitive inhibitor of uPA, 15 and amiloride. Both compounds have been shown to reduce tumor size in experimental animals [Jankun et al., *Cancer Res.* 57:559-563 (1997); Billstrom et al., *Int. J. Cancer* 61:542-547 (1995)]. Recently, epigallo-catechin-3 gallate (EGCG), a polyphenol found in green tea, was reported to 20 bind uPA and inhibit its activity [Jankun et al., *Nature* 387:561 (1997)]. Those researchers concluded EGCG is a weaker inhibitor of uPA than amiloride, but suggested EGCG can be consumed in much higher doses than amiloride without toxic effect. A competitive inhibitor of uPA, α -N- 25 benzylsulfonyl-p-aminophenylalanine, is disclosed by Pye et al. in U.S. Patent No. 4,165,258.

Other approaches at inhibiting the uPA/uPAR system include development of a bifunctional hybrid molecule consisting of the uPAR-binding domain of uPA and PAI-2, 30 which is said to inhibit uPA and bind uPAR *in vitro* [Ballance et al., *Eur. J. Biochem.* 207:177-183 (1992)]. Antagonists of uPAR also have been studied [Doyle and

Rosenberg, U.S. Patent No. 5,656,726; Min et al., Cancer Res. 56:2428-2433 (1996)], as have antisense oligonucleotides complementary to uPA [Wilhelm et al., Clin. Exp. Metast. 13:296-302 (1995); Iversen and Scholar, U.S. 5 Patent No. 5,552,390]. Antibodies directed against uPAR, and said to inhibit the binding of uPA to UPAR, are disclosed by Dano et al. in U.S. Patent No. 5,519,120. Small molecules said to inhibit urokinase, along with a variety of other serine proteases, include those disclosed 10 by Abe et al. in U.S. Patent No. 5,508,385 and U.S. Patent No. 5,153,176, and by Takano et al. in J. Pharmacol. Exp. Therapeut. 271:1027-1033 (1994).

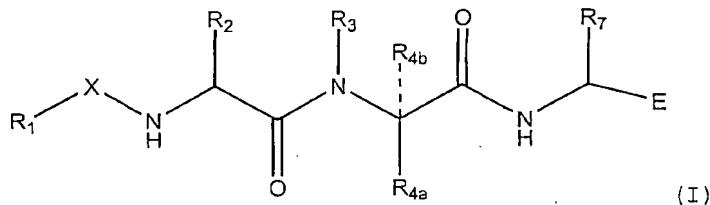
Compounds have been developed to directly inhibit the binding of u-PA to uPAR (Crowley et al., Proc. Natl. Acad. 15 Sci. USA 90:5021-5025 (1993); Goodson et al., Proc. Natl. Acad. Sci. USA 91:7129-7133 (1994); Kobayashi et al., Brit. J. Cancer 67:537-544 (1993), and Int. J. Cancer 57:727-73f3 (1994), and J. Biol. Chem. 270:8361-8366 (1995); Lu et al., FEBS Lett. 356:56-59 (1994) and FEBS Lett. 380:21-24 20 (1996)].

Additionally, pro-hepatocyte growth factor (HGF), a cell migration stimulating protein, is a substrate of uPA [Naldinie et al., EMBO J. 11:4825-4833 (1992)]. Direct cleavage of a 66kDa extracellular matrix protein and 25 fibronectin by uPA also has been reported, which suggests a more direct role for uPA in facilitating cell migration [Quigley et al., Proc. Natl. Acad. Sci. 84:2776-2780 (1987)]. Thus, inhibition of uPA may affect these activities, as well.

Summary of the Invention

The present invention is directed to novel peptidic non-covalent urokinase inhibitors. The compounds have an arginine mimic at P1. These compounds have activity as potent inhibitors of urokinase and thereby are useful in decreasing its deleterious effects. Compounds of the present invention are active in inhibiting blood vessel formation, particularly that related to a pathologic process.

Thus in one aspect, the present invention is directed to compounds of the formula (I):



wherein:

- (a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, $-(C=O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R')-$, and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with the proviso that when X is $-P(O)(R')-$, then R' is not hydrogen;
- (b) R₁ is selected from the group consisting of:
- (1) alkyl of 1 to about 12 carbon atoms which is unsubstituted or substituted with 1 or 2 substituents
- selected from the group consisting of Y₁ and Y₂,
- (2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 3 to about 8 carbon

atoms which is unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(3) cycloalkyl of 3 to about 15 carbon atoms,
5 which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(4) heterocycloalkyl of 4 to about 10 ring atoms
with the ring atoms selected from carbon and heteroatoms,
10 wherein the heteroatoms are selected from the group
consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1
or 2, which is unsubstituted or mono-, di-, or tri-
substituted on the ring with 1 to 3 substituents selected
from the group consisting of Y₁, Y₂, and Y₃,

15 (5) heterocyclo of 4 to about 10 ring atoms with
the ring atoms selected from carbon and heteroatoms, wherein
the heteroatoms are selected from the group consisting of
oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2,

including, -N_v wherein -N_v is a 5 to 7 member
20 heterocycle having 3 to 6 ring carbon atoms, where V is
-CH₂- , -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted or
mono-, di-, or tri-substituted on the ring carbons with 1 to
3 substituents selected from the group consisting of Y₁, Y₂,
and Y₃,

25 (6) alkenyl of 2 to about 6 carbon atoms which is
unsubstituted or substituted with cycloalkyl of about 3 to
about 8 carbon atoms, which is unsubstituted or mono-, di-,
or tri-substituted on the ring with 1 to 3 substituents
selected from the group consisting of Y₁, Y₂, and Y₃,

30 (7) aryl of about 6 to about 14 carbon atoms
which is unsubstituted or mono-, di- or tri-substituted with

1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

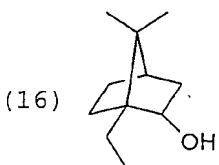
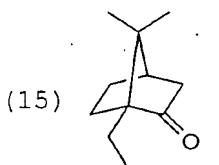
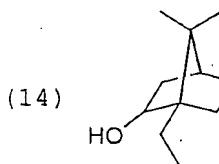
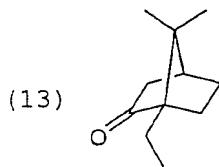
(9) aralkyl of about 7 to about 15 carbon atoms which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(11) aralkenyl of about 8 to about 16 carbon atoms which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted on the ring carbons with 1 to

3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,



(17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms,

10 (18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(19) perfluoroaryl of about 6 to about 14 carbon atoms,

(20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

15 (21) hydrogen when X is a direct link; wherein each Y₁, Y₂, and Y₃ is independently selected and is

20 (i) selected from the group consisting of halogen, cyano, nitro, tetrazolyl, guanidino, amidino, methylguanidino, -CF₃, -CF₂CF₃, -CH(CF₃)₂, -C(OH)(CF₃)₂, -OCF₃, -OCF₂H, -OCF₂CF₃, -OC(O)NH₂, -OC(O)NHZ₁, -OC(O)NZ₁Z₂,

-NHC(O)Z₁, -NHC(O)NH₂, -NHC(O)NZ₁, -NHC(O)NZ₁Z₂, -C(O)OH,
-C(O)OZ₁, -C(O)NH₂, -C(O)NHZ₁, -C(O)NZ₁Z₂, -P(O)₃H₂,
-P(O)₃(Z₁)₂, -S(O)₃H, -S(O)_mZ₁, -Z₁, -OZ₁, -OH, -NH₂, -NHZ₁,
-NZ₁Z₂, -C(=NH)NH₂,

5 -C(=NOH)NH₂, -N-morpholino, and -S(O)_m(CF₂)_qCF₃, wherein m is
0, 1 or 2, q is an integer from 0 to 5, and Z₁ and Z₂ are
independently selected from the group consisting of alkyl of
1 to about 12 carbon atoms, aryl of about 6 to about 14
carbon atoms, heteroaryl of about 5 to about 14 ring atoms,
10 aralkyl of about 7 to about 15 carbon atoms, and
heteroaralkyl of about 5 to about 14 ring atoms, or

15 (ii) Y₁ and Y₂ are selected together to be
-O[C(Z₃)(Z₄)]_rO- or -O[C(Z₃)(Z₄)]_{r+1}-, wherein r is an integer
from 1 to 4 and Z₃ and Z₄ are independently selected from the
group consisting of hydrogen, alkyl of 1 to about 12 carbon
atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl
of about 5 to about 14 ring atoms, aralkyl of about 7 to
about 15 carbon atoms, and heteroaralkyl of about 5 to about
14 ring atoms;

20 (c) R₂ is selected from the group consisting of -CH₃,
-C₂H₅, -(CH₂)₂OH, -(CH₂)₂OA₁, -CH(R₅)OH, -CH(R₅)OA₁ and
-CH₂NH-X'-R₆ wherein A₁ is -C(=O)OR₆, -C(=O)R₆ or -C(=O)NR₅R₆;
X' is selected from the group consisting of -S(O)₂-,
-S(O)₂-N(R'')-, -(C=O)-, -C(=O)-O-, -C(=O)-NH-, -P(O)(R'')-,
25 and a direct link, wherein R'' is hydrogen, alkyl of 1 to
about 4 carbon atoms, aryl of about 6 to about 14 carbon
atoms or aralkyl of about 7 to about 16 carbon atoms with
the proviso that when X' is -P(O)(R'')-, then R'' is not
hydrogen; R₅ is selected from the group consisting of:

30 (1) alkyl of 1 to about 4 carbon atoms, which is
unsubstituted or substituted with 1 to 2 substituents
selected from the group consisting of Y₁ and Y₂,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 6 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(3) cycloalkyl of 3 to about 6 carbon atoms, which is unsubstituted or mono-, di-, or trisubstituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(4) heterocycloalkyl of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(5) heterocyclo of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2,

including -N(^v), wherein -N(^v) is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted or mono-, di-, or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(6) alkenyl of 2 to about 6 carbon atoms which is unsubstituted or substituted with cycloalkyl of 3 to about 6 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(7) phenyl which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

5 (8) heteroaryl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

10 (9) alkyl of 1 to about 4 carbon atoms substituted with phenyl and which is unsubstituted or mono-, di-, or tri-substituted on the phenyl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

15 (10) heteroaralkyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and 20 which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

25 (11) aralkenyl of about 8 to about 12 carbon atoms which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

30 (12) heteroaralkenyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted on the ring carbons with 1 to

3 substituents selected from the group consisting of Y₁, Y₂, and Y₃, and

(13) hydrogen; and

R₆ is selected from the group consisting of:

5 (1) alkyl of 1 to about 12 carbon atoms, which is unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of Y₁, and Y₂,

10 (2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 8 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

15 (3) cycloalkyl of 3 to about 15 carbon atoms, which is unsubstituted or mono-, di-, or trisubstituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

20 (4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

25 (5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including -N_v, wherein -N_v is a 5 to 7 member

30 heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted

or mono-, di-, or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(6) aryl of about 6 to about 14 carbon atoms
5 which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and
10 heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(8) aralkyl of about 7 to about 15 carbon
15 atoms which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and
25 which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃, and

(10) hydrogen, with the proviso that R₆ is not hydrogen when A₁ is -C(=O)OR₆;

30 (d) R₃ is selected from H or methyl, or R₃, and R_{4a} and R_{4b} are selected together as set forth in (f);

(e) (i) R_{4a} is in the S configuration and is selected from the group consisting of H, $-CH_2-S-CH_3$, $-CH_2OH$, $-CH_2CN$, lower alkyl of 1 to about 3 carbon atoms, $-CH_2C\equiv CH$, $-CH_2CH=CH_2$ and $-CH=CH_2$ and R_{4b} is hydrogen;

5 (ii) R_{4a} and R_{4b} are independently lower alkyl of 1 to 3 carbon atoms;

(iii) R_{4a} and R_{4b} are selected together and are $-(CH_2)_k-$ wherein k is 5 or 6 to give a spirocycloalkyl group; or

10 (iv) R_3 , and R_{4a} and R_{4b} are selected together as set forth in (f);

(f) alternatively, R_3 and R_4 are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecolyl, 15 azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 4-aminoprolyl, 4- $(-CH_2NH_2)$ -prolyl, 3,4-methanoprolyl and 3,4-dehydroprolyl and R_{4b} is hydrogen;

(g) R_7 is hydrogen or alkyl of 1 to about 4 carbon atoms; and

20 (h) E is Q-T

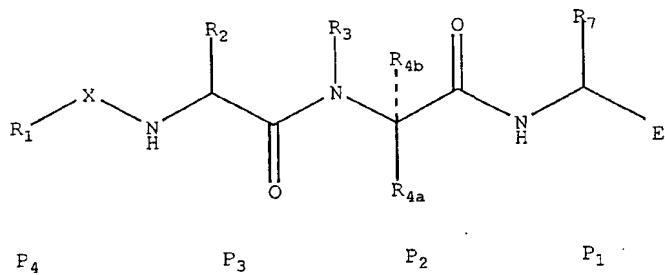
wherein (i) Q is selected from the group consisting of $-C(R_{13}R_{14})_t-$, phenyl substituted with R_8 and R_9 , a 5- or 6-membered heterocyclic ring having 1 to 2 heteroatoms substituted with R_8 or R_8 and R_9 , and a 9- or 10-membered 25 heterocyclic ring having 1 to 2 heteroatoms substituted with R_8 and R_9 , wherein heteroatoms are selected from nitrogen and sulfur; and

(ii) T is selected from the group consisting of $-C(=NR_{10})NHR_{11}$, $-NH-C(=NR_{10})NHR_{11}$ and $-NHR_{15}$;

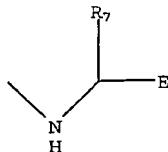
30 wherein R_8 and R_9 are independently selected from the group consisting of hydrogen, hydroxy, halogen, alkyl of 1 to about 4 carbon atoms, alkyl of 1 to about 4 carbon atoms

substituted with alkoxy of 1 to about 4 carbon atoms, alkoxy of 1 to about 6 carbon atoms, and trifluoromethyl; R₁₀ and R₁₁ are independently hydrogen, hydroxy, alkoxy of 1 to about 3 carbon atoms, trihydrocarbylsilyl of 3 to about 16 carbon atoms, alkyl of 1 to about 3 carbon atoms or -C(=O)R₁₂ with the proviso that R₁₀ and R₁₁ are not both hydroxy or alkoxy; R₁₂ is hydrogen, alkyl of 1 to about 6 carbon atoms, alkoxy of 1 to about 6 carbon atoms or (CF₂)_jCF₃ wherein j is 0, 1, 2 or 3; each of R₁₃ and R₁₄ is independently selected from the group consisting of hydrogen and lower alkyl of 1 to about 3 carbon atoms; R₁₅ is selected from the group consisting of hydrogen, alkyl of 1 to about 6 carbon atoms and -(CF₂)_nCF₃ wherein h is 0, 1, 2 or 3 and t is an integer from 0 to 6; and pharmaceutically acceptable salts thereof.

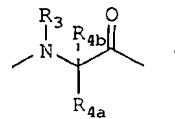
The compounds of the present invention can be divided into parts termed P₁, P₂, P₃ and P₄ as shown in the following formula Ia:



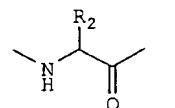
wherein X, R₁, R₂, R₃, R₄, R₇ and E are as defined in connection with formula (I). Thus, the portion of a compound of formula (I) referred to as P₁ or P1 is the moiety



The portion of a compound of formula (I) referred to as P₂ or P₂ is the moiety



The portion of a compound of formula (I) referred to as P₃ or
5 P₃ is the moiety



Among other factors, the present invention is based on our finding that the novel compounds of our invention are active as inhibitors of urokinase. Compounds of the present
10 invention exhibit activity in inhibiting angiogenesis.

In another aspect, the present invention is directed to pharmaceutical compositions comprising a therapeutically effective amount of a compound of the present invention and a pharmaceutically acceptable carrier.

15 In yet another aspect, the present invention is directed to methods of using the compounds and pharmaceutical compositions of the present invention for inhibition of urokinase.

Definitions

20 In accordance with the present invention and as used herein, the following terms are defined to have following meanings, unless explicitly stated otherwise:

The term "alkenyl" refers to unsaturated aliphatic groups having at least one double bond.

25 The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and cyclic (including polycyclic) groups.

The terms "alkoxy" and "alkoxyl" refer to a group having the formula, R-O-, wherein R is an alkyl group.

The term "alkoxycarbonyl" refers to -C(O)OR wherein R is alkyl.

5 The term "aralkenyl" refers to an alkenyl group substituted with an aryl group. Preferably the alkenyl group has from 2 to about 6 carbon atoms.

10 The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl, phenethyl, and the like, all of which may be optionally substituted. Preferably the alkyl group has from 1 to about 6 carbon atoms.

15 The term "aryl" refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes a carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

The term "aryloxy" refers to a group having the formula, R-O-, wherein R is an aryl group.

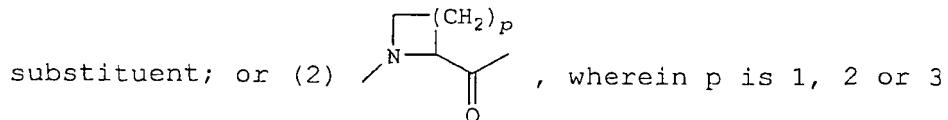
20 The term "aralkoxy" refers to a group having the formula, R-O-, wherein R is an aralkyl group.

25 The term "amino acid" refers to both natural, unnatural amino acids in their D and L stereo isomers if their structures allow such stereoisomeric forms, and their analogs. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-amino adipic acid, 3-amino adipic acid, beta-alanine,

aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4 diaminoisobutyric acid, demosine, 5 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, 10 ornithine and pipecolic acid. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, 15 S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been 20 chemically modified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

25 The term "amino acid residue" refers to radicals having the structure: (1) -C(O)-R-NH-, wherein R typically is -CH(R')-, wherein R' is H or a carbon containing



representing the azetidinecarboxylic acid, proline or 30 pipecolic acid residues, respectively.

"Biaryl" refers to phenyl substituted by carbocyclic or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring.

5 "Brine" refers to an aqueous saturated solution of sodium chloride.

"Carbocyclic aryl" refers to aromatic groups wherein the ring atoms on the aromatic ring are carbon atoms.

Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and naphthyl groups, all of which may be optionally 10 substituted. Suitable carbocyclic aryl groups include phenyl and naphthyl. Suitable substituted carbocyclic aryl groups include indene and phenyl substituted by one to two substituents such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkoxy carbonyl, halogen, 15 trifluoromethyl, difluoromethyl, nitro, and cyano.

Substituted naphthyl refers to naphthyl, more preferably 1- or 2-naphthyl, substituted by Y₁, Y₂ and/or Y₃ as defined in connection with formula (I) hereinabove.

20 "Cycloalkenyl" refers to a cyclic alkenyl group. Suitable cycloalkenyl groups include, for example, cyclopentenyl and cyclohexenyl.

25 "Cycloalkyl" refers to a cyclic alkyl group having at least one ring and includes polycyclic groups, including fused ring cyclic alkyl groups. Suitable cycloalkyl groups include, for example, cyclohexyl, cyclopropyl, cyclopentyl, and cycloheptyl.

"Cyclohexylmethyl" refers to a cyclohexyl group attached to CH₂.

30 "Fused carbocyclic" refers to a multicyclic fused carbocyclic ring having both aromatic and non-aromatic rings. Suitable fused carbocyclic rings include fluorenyl, tetralin and the like.

"Fused carbocyclic alkyl" refers to an alkyl group substituted with a fused carbocyclic ring moiety, preferably a multicyclic fused carbocyclic ring including both aromatic and non-aromatic rings. Suitable fused carbocyclic alkyl groups include fluorenylmethyl, and the like.

The term "halogen" refers to fluorine, chlorine, bromine and iodine.

"Heteroaralkenyl" refers to an alkenyl group substituted with a heteroaryl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkenyl group has from 2 to about 6 carbon atoms.

"Heteroaralkyl" refers to an alkyl group substituted with a heteroaryl, such as picolyl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkyl group has from 1 to about 6 carbon atoms.

"Heteroaryl" refers to aromatic groups having from 1 to 14 carbon atoms and the remainder of the ring atoms are heteroatoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Suitable heteroatoms include oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, and suitable heterocyclic aryls

include furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, imidazolyl, and the like.

"Heterocyclo" refers to a reduced heterocyclic ring system comprised of carbon, nitrogen, oxygen and/or sulfur atoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

"Heterocycloalkyl" refers to an alkyl group substituted with a heterocyclo group, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkyl group has from about 1 to about 6 carbon atoms.

The term "lower" referred to herein in connection with organic radicals or groups defines such radicals or groups with one and up to and including 5 carbon atoms, preferably up to and including 4 carbon atoms, and advantageously one or two carbon atoms. Such radicals or groups may be straight chain or branched chain.

"Perfluoroalkyl" refers to an alkyl group which has every hydrogen replaced with fluorine.

"Perfluoroaryl" refers to an aryl group which has every hydrogen replaced with fluorine.

"Perfluoroarylalkyl" refers to an aralkyl group in which every hydrogen on the aryl moiety is replaced with fluorine.

"Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the

combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

- "AcN", CH₃CN or "MeCN" refers to acetonitrile.
"AIBN" refers to 2, 2'-azobisisobutyronitrile.
"Bn" refers to benzyl.
10 "Boc" refers to t-butoxycarbonyl.
"Boc₂O" refers to Boc anhydride (di-tert-butyl carbonate).
"BOC-ON" refers to 2-(tert-butoxycarbonyloxyamino)-2-phenylacetonitrile.
15 "BzlSO₂" refers to benzylsulfonyl.
"Cbz" or "CBz" refers to benzyloxycarbonyl.
"CNNH₂" or "H₂N" refers to cyanamide.
"CsCo₃" refers to cesium carbonate.
"DCA" refers to dichloroacetic acid.
20 "DCC" refers to N,N'-dicyclohexylcarbodiimide.
"DCM" or "CH₂Cl₂" refers to dichloromethane.
"DIEA" refers to diisopropylethylamine.
"DMF" refers to N,N-dimethylformamide.
"DMSO" refers to dimethyl sulfoxide.
25 "DMAP" refers to 4-N,N-dimethylaminopyridine.
"EDC" refers to 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride salt.
"Et₃N" or "TEA" refers to triethylamine.
"EtOAc" refers to ethyl acetate.
30 "EtOH" refers to ethanol.
"HATU" refers to O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethylureonium hexafluorophosphate.

"HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

"HCl" refers to hydrochloric acid.

"HOAc" refers to acetic acid.

5 "HOAt" or "HOAT" refers to 1-hydroxy-7-azabenzotriazole.

"HOBt" refers to 1-hydroxybenzotriazole monohydrate.

"i-BuOCOCl" refers to isobutylchloroformate.

"HPLC" refers to high pressure liquid chromatography.

10 "LiAlH₄" refers to lithium aluminum hydride.

"LiAlH₂(OEt)₂" refers to lithium aluminum hydride diethoxide.

"Me" refers to methyl.

"MeOH" refers to methanol.

15 "NMM" refers to N-methylmorpholine.

"NBS" refers to N-bromosuccinimide.

"PhB(OH)₂" refers to phenylboronic acid.

"Ph₃P" or "PPh₃" refers to triphenylphospine.

20 "PyBOP" refers to benzotriazole-ly-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.

"RP-HPLC" refers to reverse phase high pressure liquid chromatography.

"TFA" refers to trifluoroacetic acid.

"THF" refers to tetrahydrofuran.

25 "TLC" refers to thin layer chromatography.

Brief Description Of The Drawings

Figure 1 depicts a reaction scheme for solution phase synthesis of an intermediate useful in synthesizing a compound of the present invention. Compound 1-1 is N- α -Cbz-D-serine (O-t-butyl), compound 1-2 is alanine methyl ester, hydrochloride salt. In this figure, "i" through "iv" are

defined as i) EDC, 1-hydroxybenzotriazole and acetonitrile, diisopropylethylamine to give Cbz-D-Ser (O-t-butyl)-Ala-OMe; (ii) ethanol/acetic acid/water (4:1:1), 10% Pd on carbon, 45 psi H₂ for 2 hours, 95% yield after work-up; iii) 5 acetonitrile, benzenesulfonyl chloride, diisopropylethylamine, 43% yield after work-up; and iv) methanol, 1.0 M lithium hydroxide, acidification on DOWEX ion exchange resin, eluting with methanol/water, 95% yield after work-up. See also Examples 60 to 62.

10 Figure 2 depicts a reaction scheme for a solution phase synthetic route which may be used to prepare an intermediate useful in the preparation of a compound of the present invention. In this figure, "i" through "iii" are defined as: i) isobutyl chloroformate, sodium carbonate, water, 99.5% yield after workup; ii) alanine t-butyl ester, hydrochloride salt, EDC, and hydroxybenzotriazole in acetonitrile; diisopropylethylamine, quantitative yield after workup; and iii) TFA, DCM, quantitative yield after workup. See also Examples 74 to 76.

15 20 Figure 3 depicts a reaction scheme for the synthesis of intermediates which may be used in the preparation of compounds of the present invention. In this figure, "i" through "xi" are defined as follows: i) CuCN, DMF, reflux (4 hours); ii) EtOAc, 10% aqueous NaCN; iii) N-bromosuccinimide, 25 2,2'-azo-bisisobutyronitrile, CCl₄, reflux (5 hours); iv) NaN₃, DMF; v) triphenylphosphine, THF, water, O C, stirring (10 hours); vi) K₂CO₃, Boc₂O, water, dioxane; vii) 30 hydroxylamine HCl, NMM, MeOH; viii) 10% Pd/C, MeOH, 45 psi H₂ (10 hours); ix) 4M HCl in dioxane; x) CsCO₃, iodopropane in DMF; and xi) 4M HCl, dioxane, 3 hours, room temperature.

Figure 4 depicts a reaction scheme for the synthesis of intermediates which may be used in the preparation of

compounds of the present invention. In this figure, "i" through "iv" are defined as follows: i) NaN₃, DMF; ii) 10% Pd/C, EtOAc, 45 psi H₂ (11 hours); iii) hydroxylamine HCl, NMM, MeOH; and iv) 10% Pd/C, MeOH, 45 psi H₂ (48 hours).

5 Figure 5 depicts a reaction scheme for the synthesis of intermediates which may be used in the preparation of compounds of the present invention. In this figure, "i" through "vii" are defined as follows: i) Cu(I)CN, DMF; ii) NBS, benzoylperoxide, CCl₄, 80°C (14 hours); iii) NaN₃, DMF, stirring (20 hours); iv) hydroxylamine HCl, NMM, MeOH, stirring (3 days); v) CsCO₃, iodopropane, DMF, 50°C (20 hours); vi) triphenylphosphine, THF, stirring (20 hours); and vii) 3M NaOH to pH 14.

10 Figure 6 depicts a reaction scheme for the synthesis of a compound of the present invention where R₂ is -CH₂O A₁ and A₁ is -C(=O)R₆, using as an intermediate, compound of Example 9. In this figure, "i" is defined as: i) pyridine, R₆COCl.

15 Figure 7 depicts a reaction scheme for the synthesis of certain compounds of the present invention. In this figure, "i" through "vi" are defined as follows: i) trifluoracetic anhydride, 0°C, stir overnight; ice, CH₂Cl₂, Na₂SO₄; ii) Pd/C (10%) in MeOH (overnight); iii) N-N'-di-Boc-N"-trifluoromethanesulfonyl-guanidine, TEA, CH₂Cl₂, 6 hours; HCl, brine, Na₂SO₂; column chromatography (CH₂Cl₂/MeOH 99:1); iv) potassium carbonate, H₂O/MeOH (2:15), overnight; CH₂Cl₂/MeOH (9:1), Na₂SO₄; v) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, Acetonitrile, overnight; EtOAc, HCl, NaHCO₃, brine; HPLC (CH₃CN, H₂O, 0.1% TFA); vi) CH₂Cl₂/TFA (1:1), 90 minutes; HPLC (CH₃CN, H₂O, 0.1% TFA).

20 Figure 8 depicts the reaction scheme for the synthesis of certain compounds of the present invention. In this

figure "i" through "v" are defined as: i) trifluoroacetic anhydride, stir overnight; ice, CH₂Cl₂, Na₂SO₄; ii) Pd/C (10%) in MeOH (overnight); iii) N-N'-Boc-N''-trifluoromethanesulfonyl-guanidine, TEA, CH₂Cl₂, 24 hours; 5 HCl, brine, Na₂SO₄; column chromatography (CH₂Cl₂/MeOH 98:2); iv) potassium carbonate, H₂O/MeOH (1:1), overnight; H₂O, CH₂Cl₂/MeOH (9:1), Na₂SO₄; and v) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, in acetonitrile, room temperature overnight; EtOAc, HCl, NaHCO₃, brine; CH₂Cl₂/TFA 10 (1:1), room temperature, 2 hours, HPLC (CH₃CN, H₂O, 0.1% TFA).

Figure 9 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "vi" are defined as follows: i) trifluoroacetic anhydride, 0°C, one hour; ii) KNO₃, -20°C, stir overnight; 15 CH₂Cl₂, Na₂SO₄, column chromatography; iii) HCl in MeOH, 0°C, SnCl₂, stir 30 minutes, NaHCO₃, CH₂Cl₂, Na₂SO₄; iv) N₁N'-di-Boc-N''-trifluoromethanesulfonyl-guanidine, TEA, CH₂Cl₂, stirring 24 hours; HCl (1M), brine, Na₂SO₄, column chromatography; v) K₂CO₃, H₂O/MeOH (1:1), stir overnight; H₂O, 20 CH₂Cl₂/MeOH (95:5), Na₂SO₄; and vi) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, AcN, stir overnight; EtOAc, HCl (1M), aqueous NaHCO₃, brine, Na₂SO₄; HPLC.

Figures 10A to 10F depict certain preferred compounds 25 of the present invention.

Figure 11 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "xii" are defined as follows: i) CCl₄, N-bromosuccinimide; N₂, AIBN, stirring, flash chromatography; 30 ii) DMF, NaN₃, stir overnight, diethylether, water, brine, MgSO₄, filter; (iii) MeOH, TEA, 65°C, 4 hours; EtOAc, H₂O, brine, MgSO₄; iv) THF/water, Ph₃P, stir overnight; 1N HCl,

H₂O, DCM, pH~9; v) dioxane/water, K₂CO₃, Boc₂O, stir overnight; EtOAc, aqueous NaHCO₃, brine, Na₂SO₄, flash column chromatography; vi) DMF, 2-iodopropane, CsCO₃; EtOAc, aqueous NaHCO₃, Na₂SO₄, flash column chromatography; vii) dioxane, 4N HCl in dioxane, solvent removal; viii) AcN, DIEA; Boc-alanine, EDC, HOEt, stir overnight; EtOAc, aqueous NaHCO₃, brine, Na₂SO₄, flash column chromatography; ix) dioxane, 4N HCl in dioxane, remove solvent, x) AcN, DIEA, BnSO₂-dSer(tBu)-OH, EDC, HOEt, stir overnight; EtOAc, aqueous NaHCO₃, brine, Na₂SO₄, RP-HPLC; xc) DCM, TFA; RP-HPLC; and xii) H₂O, HOAc, Zn dust; RP-HPLC.

Figure 12 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "vi" are defined as follows: i) EDC, HOEt, DIEA and CH₃CN; ii) TFA, CH₂Cl₂; iii) BNSO₂-dSer(tBu)-OH, EDC, HOEt, 2,4,6-collidine, CH₃CN; iv) hydroxylamine hydrochloride; v) Zn/acetic acid; and vi) TFA, CH₂Cl₂.

Figures 13A to 13C depict certain preferred compounds of the present invention.

Figure 14 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "v" are defined as follows: i) BH₃, THF, 78% yield; ii) BOC-ON, 95% yield; iii) Pd/C, H₂, 81% yield; iv) CNBr, NaHCO₃, CH₃CN, H₂O; v) TFA, CH₂Cl₂; and vi) benzylsulfonyl-D-Serine-L-alanine carboxylate, HATU, HOAT, DIEA, CH₃CN.

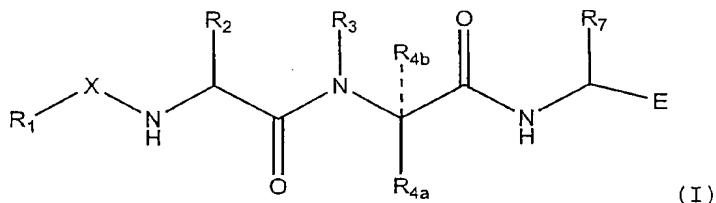
Figure 15 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "iii" are defined as follows: i) thionylchloride, MeOH; ii) Na/Hg (5%), CNNH₂, reflux, 62% yield; and iii) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, CH₃CN.

Figure 16 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "vi" are defined as follows: i) BH_3 , THF; ii) $\text{Cl}/\text{Dioxane}$, quantitative yield; iii) BOC-ON, THF; iv) TEA, CH_2Cl_2 ; v) TFA/ CH_2Cl_2 ; and vi) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, CH_3CN .

Figure 17 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "vi" are defined as follows: i) BH_3 , THF; ii) HCl, quantitative yield; iii) BOC-ON, THF; iv) TEA, CH_2Cl_2 ; v) TFA, CH_2Cl_2 ; and vi) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, CH_3CN .

Detailed Description of the Invention

15



wherein:

(a) X is selected from the group consisting of $-\text{S}(\text{O})_2-$, $-\text{N}(\text{R}')-\text{S}(\text{O})_2-$, $-(\text{C}=\text{O})-$, $-\text{OC}(\text{=O})-$, $-\text{NH}-\text{C}(\text{=O})-$, $-\text{P}(\text{O})(\text{R}')-$, and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with the proviso that when X is $-\text{P}(\text{O})(\text{R}')-$, then R' is not hydrogen;

(b) R_1 is selected from the group consisting of:

- (1) alkyl of 1 to about 12 carbon atoms which is unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of Y₁ and Y₂,
- (2) alkyl of 1 to about 3 carbon atoms
- 5 substituted with cycloalkyl of about 3 to about 8 carbon atoms which is unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,
- (3) cycloalkyl of 3 to about 15 carbon atoms,
- 10 which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,
- (4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms,
- 15 wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,
- 20 (5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including, -N(_v) wherein -N(_v) is a 5 to 7 member
- 25 heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted or mono-, di-, or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,
- 30 (6) alkenyl of 2 to about 6 carbon atoms which is unsubstituted or substituted with cycloalkyl of about 3 to

about 8 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

- (7) aryl of about 6 to about 14 carbon atoms
5 which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

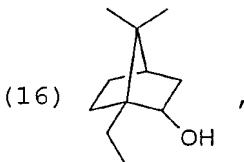
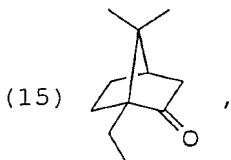
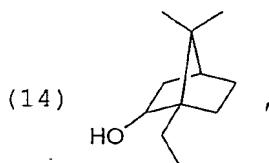
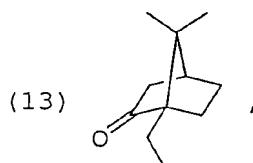
- (8) heteroaryl of about 5 to about 14 ring atoms
10 with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

- (9) aralkyl of about 7 to about 15 carbon atoms
15 which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

- 20 (10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and
25 which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

- (11) aralkenyl of about 8 to about 16 carbon atoms
30 which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or 5 mono-, di- or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,



10

(17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms,

15

(18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(19) perfluoroaryl of about 6 to about 14 carbon atoms,

20 (20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(21) hydrogen when X is a direct link;
wherein each Y₁, Y₂, and Y₃ is independently selected and is
(i) selected from the group consisting of
halogen, cyano, nitro, tetrazolyl, guanidino, amidino,
5 methylguanidino, -CF₃, -CF₂CF₃, -CH(CF₃)₂, -C(OH)(CF₃)₂, -OCF₃,
-OCF₂H, -OCF₂CF₃, -OC(O)NH₂, -OC(O)NZ₁Z₂, -OC(O)NZ₁Z₂,
-NHC(O)Z₁, -NHC(O)NH₂, -NHC(O)NZ₁, -NHC(O)NZ₁Z₂, -C(O)OH,
-C(O)OZ₁, -C(O)NH₂, -C(O)NZ₁, -C(O)NZ₁Z₂, -P(O)₃H₂,
-P(O)₃(Z₁)₂, -S(O)₃H, -S(O)_mZ₁, -Z₁, -OZ₁, -OH, -NH₂, -NHZ₁,
10 -NZ₁Z₂, -C(=NH)NH₂,
-N-morpholino, and -S(O)_m(CF₂)_qCF₃, wherein m is 0, 1 or 2, q
is an integer from 0 to 5, and Z₁ and Z₂ are independently
selected from the group consisting of alkyl of 1 to about 12
carbon atoms, aryl of about 6 to about 14 carbon atoms,
15 heteroaryl of about 5 to about 14 ring atoms, aralkyl of
about 7 to about 15 carbon atoms, and heteroaralkyl of about
5 to about 14 ring atoms, or
(ii) Y₁ and Y₂ are selected together to be
-O[C(Z₃)(Z₄)]_rO- or -O[C(Z₃)(Z₄)]_{r+1}-, wherein r is an integer
20 from 1 to 4 and Z₃ and Z₄ are independently selected from the
group consisting of hydrogen, alkyl of 1 to about 12 carbon
atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl
of about 5 to about 14 ring atoms, aralkyl of about 7 to
about 15 carbon atoms, and heteroaralkyl of about 5 to about
25 14 ring atoms;
(c) R₂ is selected from the group consisting of -CH₃,
-C₂H₅, -(CH₂)₂OH, -(CH₂)₂OA₁, -CH(R₅)OH, -CH(R₅)OA₁ and
-CH₂NH-X'-R₆ wherein A₁ is -C(=O)OR₆, -C(=O)R₆ or -C(=O)NR₅R₆;
X' is selected from the group consisting of -S(O)₂-,
30 -S(O)₂-N(R")-, -(C=O)-, -C(=O)-O-, -C(=O)-NH-, -P(O)(R")-,
and a direct link, wherein R" is hydrogen, alkyl of 1 to
about 4 carbon atoms, aryl of about 6 to about 14 carbon

atoms or aralkyl of about 7 to about 16 carbon atoms with the proviso that when X' is $-P(O)(R'')-$, then R'' is not hydrogen; R₅ is selected from the group consisting of:

(1) alkyl of 1 to about 4 carbon atoms, which is 5 unsubstituted or substituted with 1 to 2 substituents selected from the group consisting of Y₁ and Y₂,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 6 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on 10 the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(3) cycloalkyl of 3 to about 6 carbon atoms, which is unsubstituted or mono-, di-, or trisubstituted on the ring with 1 to 3 substituents selected from the group 15 consisting of Y₁, Y₂, and Y₃,

(4) heterocycloalkyl of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 20 or 2, which is unsubstituted or mono-, di-, or tri- substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(5) heterocyclo of 4 to about 6 ring atoms with 25 the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2,

including $-N(V)$, wherein $-N(V)$ is a 5 to 7 member 30 heterocycle having 3 to 6 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$, $-S(O)_2-$ or $-S-$, which is unsubstituted or mono-, di-, or tri-substituted on the ring carbons with 1

to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(6) alkenyl of 2 to about 6 carbon atoms which is unsubstituted or substituted with cycloalkyl of 3 to about 6
5 carbon atoms, which is unsubstituted or mono-, di-, or tri-
substituted on the ring with 1 to 3 substituents selected
from the group consisting of Y₁, Y₂, and Y₃,

(7) phenyl which is unsubstituted or mono-, di-
or tri-substituted with 1 to 3 substituents selected from
10 the group consisting of Y₁, Y₂, and Y₃,

(8) heteroaryl of about 5 to about 6 ring atoms
with the ring atoms selected from carbon and heteroatoms,
wherein the heteroatoms are selected from oxygen, nitrogen,
and sulfur, and which is unsubstituted or mono-, di- or tri-
15 substituted with 1 to 3 substituents selected from the group
consisting of Y₁, Y₂, and Y₃,

(9) alkyl of 1 to about 4 carbon atoms
substituted with phenyl and which is unsubstituted or mono-,
di-, or tri-substituted on the phenyl ring with 1 to 3
20 substituents selected from the group consisting of Y₁, Y₂,
and Y₃,

(10) heteroaralkyl of about 5 to about 6 ring
atoms with the ring atoms selected from carbon and
heteroatoms, wherein the heteroatoms are selected from
25 oxygen, nitrogen, and sulfur, and which is unsubstituted or
substituted on the alkyl chain with hydroxy or halogen and
which is unsubstituted on the ring or mono-, di- or tri-
substituted on the ring with 1 to 3 substituents selected
from the group consisting of Y₁, Y₂, and Y₃,

30 (11) aralkenyl of about 8 to about 12 carbon atoms
which is unsubstituted or mono-, di-, or tri-substituted on

the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(12) heteroaralkenyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and

5 heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃, and

10 (13) hydrogen; and

R₆ is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms,

which is unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of Y₁, and

15 Y₂,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 8 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group

20 consisting of Y₁, Y₂, and Y₃,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is unsubstituted or mono-, di-, or trisubstituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

25 (4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is unsubstituted or mono-, di-, or tri-
30 substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including -N(*v*), wherein -N(*v*) is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted or mono-, di-, or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(6) aryl of about 6 to about 14 carbon atoms which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(8) aralkyl of about 7 to about 15 carbon atoms which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and

which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃, and

(10) hydrogen, with the proviso that R₆ is not
5 hydrogen when A₁ is -C(=O)OR₆;

(d) R₃ is selected from H or methyl, or R₃, and R_{4a} and R_{4b} are selected together as set forth in (f);

10 (e) (i) R_{4a} is in the S configuration and is selected from the group consisting of H, -CH₂-S-CH₃, -CH₂OH, -CH₂CN, lower alkyl of 1 to about 3 carbon atoms, -CH₂C≡CH,
-CH₂CH=CH₂ and -CH=CH₂ and R_{4b} is hydrogen;

(ii) R_{4a} and R_{4b} are independently lower alkyl of 1 to 3 carbon atoms;

15 (iii) R_{4a} and R_{4b} are selected together and are - (CH₂)_k- wherein k is 5 or 6 to give a spirocycloalkyl group;
or

(iv) R₃, and R_{4a} and R_{4b} are selected together as set forth in (f);

20 (f) alternatively, R₃ and R₄ are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecolyl, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 4-aminoprolyl, 4-(-CH₂NH₂)-prolyl 3,4-methanoprolyl and 3,4-dehydropopylyl and R_{4b} is hydrogen;

25 (g) R₇ is hydrogen or alkyl of 1 to about 4 carbon atoms; and

(h) E is Q-T

wherein (i) Q is selected from the group consisting of - C(R₁₃R₁₄)_t-, phenyl substituted with R₈ and R₉, a 5- or 6-membered heterocyclic ring having 1 to 2 heteroatoms substituted with R₈ or R₈ and R₉ and a 9- or 10-membered heterocyclic ring having 1 to 2 heteroatoms substituted with

R₈ and R₉, wherein heteroatoms are selected from nitrogen and sulfur; and

(ii) T is selected from the group consisting of -C(=NR₁₀)NHR₁₁, -NH-C(=NR₁₀)NHR₁₁ and -NHR₁₅;

5 wherein R₈ and R₉ are independently selected from the group consisting of hydrogen, hydroxy, halogen, alkyl of 1 to about 4 carbon atoms, alkyl of 1 to about 4 carbon atoms substituted with alkoxy of 1 to about 4 carbon atoms, alkoxy of 1 to about 6 carbon atoms, and trifluoromethyl; R₁₀ and R₁₁
10 are independently hydrogen, hydroxy, alkoxy of 1 to about 3 carbon atoms, trihydrocarbylsilyl of 3 to about 16 carbon atoms, alkyl of 1 to about 3 carbon atoms or -C(=O)R₁₂ with the proviso that R₁₀ and R₁₁ are not both hydroxy or alkoxy; R₁₂ is hydrogen, alkyl of 1 to about 6 carbon atoms, alkoxy of 1 to about 6 carbon atoms or (CF₂)_jCF₃ wherein j is 0, 1, 2 or 3; each of R₁₃ and R₁₄ is independently selected from the group consisting of hydrogen and lower alkyl of 1 to about 3 carbon atoms; R₁₅ is selected from the group consisting of hydrogen, alkyl of 1 to about 6 carbon atoms and -(CF₂)_hCF₃
15 wherein h is 0, 1, 2 or 3 and t is an integer from 0 to 6;
20 and pharmaceutically acceptable salts thereof.

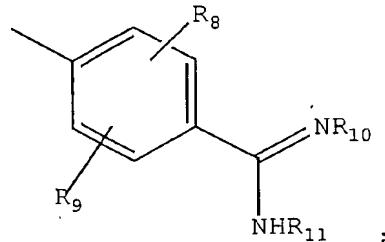
According to another aspect of the present invention, preferred compounds include those of formula (I) with the proviso that, when E is -C(R₁₃R₁₄)_t-, then T is not -NHR₁₅.

25 According to a more preferred aspect, preferred compounds of formula I include those compounds where Q is selected from the group consisting of phenyl substituted with R₈ and R₉, a 5- or 6-membered heterocyclic ring having 1 to 2 heteroatoms substituted with R₈ or R₈ and R₉ and a 9- or 10-membered
30 heterocyclic ring having 1 to 2 heteroatoms, wherein the heteroatoms are selected from the group consisting of

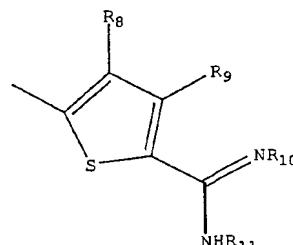
nitrogen and sulfur. More preferably, Q is phenyl substituted with R₈ and R₉. More preferably T is -C(=NR₁₀)NHR₁₁ or -NHC(=NR₁₀)NHR₁₁.

According to one aspect of the present invention,
5 preferred are compounds of formula I.
wherein E is selected from the group consisting of

(1)

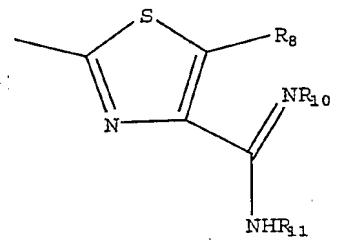


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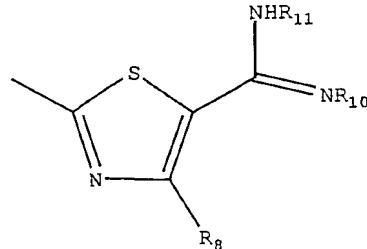


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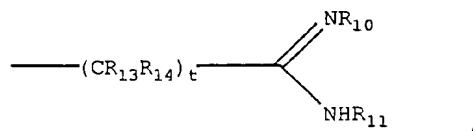
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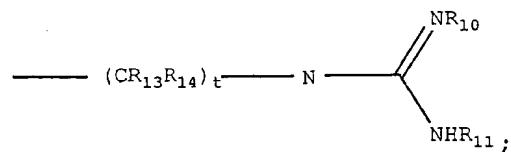
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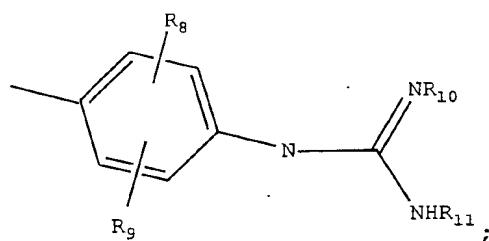


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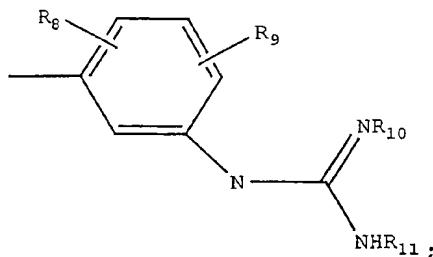
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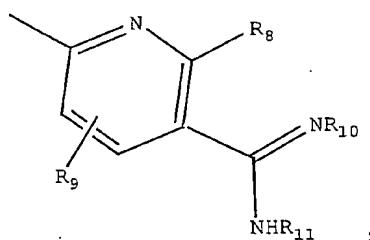


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(8)

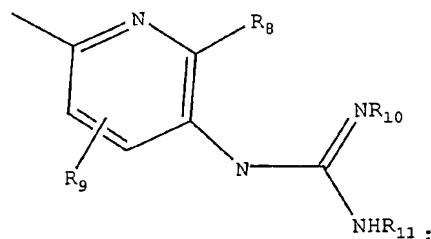


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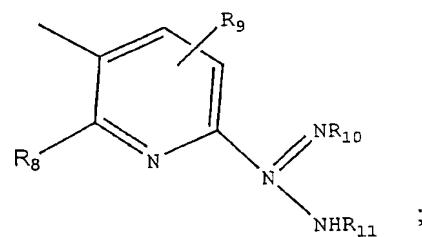


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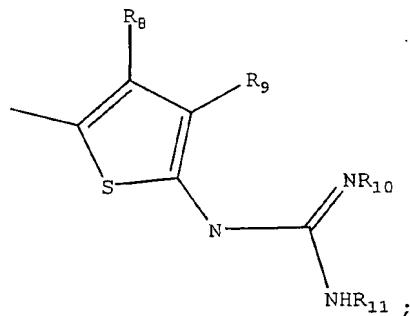


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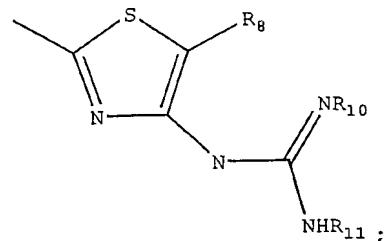
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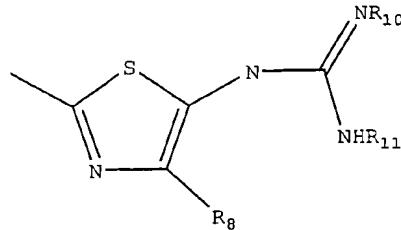


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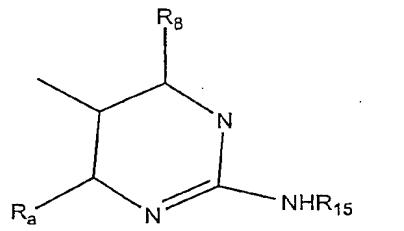
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(14)

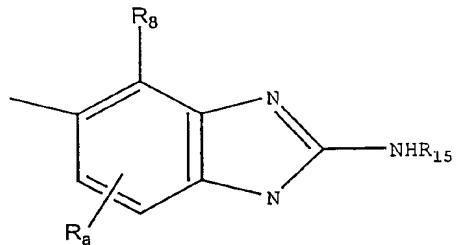


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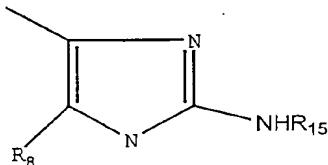
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(16)



and

(17)



10

Preferred X groups include -S(O)₂-, -OC(=O)-, -NH-C(=O)-, and a direct link. Especially preferred are -S(O)₂- and -OC(=O)-.

Preferred R₁ groups include alkyls, especially isobutyl, 2-ethylhexyl, methyl, n-butyl, isopropyl, cyclohexylmethyl, and cyclohexylpropyl; cycloalkyl, especially (-)menthyl, (+)menthyl, and cyclohexyl; aryls, especially naphthyl and phenyl; aralkyls, especially benzyl, 3-phenylpropyl, and 2-phenylethyl; and fused carbocyclic alkyls, especially fluorenethylmethyl. Especially preferred R₁ groups include phenyl, benzyl, 2-phenylethyl, isobutyl, n-butyl and 3-phenylpropyl.

Preferred combinations of R₁-X- include phenyl-S(O)₂-, benzyl-S(O)₂-, 2-phenylethyl-S(O)₂-, 3-phenylpropyl-S(O)₂-, n-butyl-S(O)₂-, benzyl-OC(=O)-, and isobutyl-OC(=O)-.

Preferred R₂ groups include -CH₃, -C₂H₅, -CH₂NH-X'-R₅ and -CH(R₅)OH, wherein R₅ is hydrogen, alkyl, especially methyl, or aralkyl. Preferred chirality at the alpha carbon is R. When chiral, preferred chirality at the beta carbon is R. Preferred R₂ groups are those that define the P₃ position as d-seryl (-CH(R₅)OH where R₅ is H), (R,R)d-allothreonyl (-CH(R₅)OH where R₅ is methyl), d-2-aminobutyryl, N-β-methyloxycarbonyl-d-2,3-diaminopropionyl (-CH₂NH-X'-R₅ where R₅ is CH₃ and X' is -(C=O)O-), N-β-(2-phenylethylcarbonyl)-d-2,3-diaminopropionyl (-CH₂NH-X-R₅ where R₅ is 2-phenylethyl and X' is -(C=O)O-), N-β-benzyloxycarbonyl-d-2,3-diaminopropionyl (-CH₂NH-X'-R₅ where R₅ is benzyl and X' is -(C=O)O-) and d-alanyl(-CH₂). Especially preferred R₂ groups are those which define P₃ as d-seryl (R₅ is H) or (R,R)d-allothreonyl (R₅ is methyl). Alternate preferred R₂ groups include -(CH₂)₂OA₁ and -CH(R₅)OA₁, more preferably -CH(R₅)OA₁; preferably R₅ is H. More preferably R₂ is selected so that P₃ is defined as an

acyl or carbonate ester of d-seryl. Compounds wherein R₂ is -(CH₂)₂OA₁ or -CH(R₅)OA₁ may act as prodrugs.

A preferred R₃ group, when R₃ and R₄ are not selected together, is hydrogen. A preferred R₄ group, when R₃ and R₄ are not selected together, is methyl, vinyl, allyl or propargyl. When R₃ and R₄ are selected together, prolyl, 3-hydroxy-prolyl, 4-hydroxyprolyl, 3,4-dehydroprolyl, 3,4-methanoprollyl, and azetidine-2-carbonyl- are preferred selections to define a group at P2.

10 Preferred R₇ groups include hydrogen.

Preferred E groups include those where Q is phenyl substituted with R₈ and R₉, pyridyl substituted with R₈ and R₉, or thienyl substituted with R₈ and T is -C(=NR₁₀)NHR₁₁ or -NH-C(=NR₁₀)NHR₁₁.

15 Preferred E groups include 4-amidinophenyl, 4-guanidinophenyl, 3-amidinopropyl, and 5-(2-amidino-thienyl). Especially preferred E groups include 4-amidinophenyl and 4-guanidinophenyl.

Among the compounds of the present invention, preferred compounds include those having an R₂ element that defines d-serine or d-allothreonine or an acyl or carbonate ester thereof at the P3 position of the compound and an amidinophenyl, guanidinophenyl or amidinothienyl group at P1. Especially preferred are such compounds also having either i) a hydrogen at R₃ and methyl at R₄ (P2 is alanine), or ii) having R₃ and R₄ selected together so that P2 is prolyl, azetidine-2-carbonyl, 3,4-methanoprollyl or 3,4-dehydroprolyl.

Preferred compounds of the present invention include those depicted in Figures 10A to 10F and Figures 13A to 13C. Especially preferred are Compounds D, F, I, J, K, L, O, R,

T, U, V, AE, AH, AJ, AN and AV of Figures 10A to 10F and BG, BJ, BK and BQ of Figures 13A to 13C.

Also especially preferred are the following compounds of formula (I): compounds AX ($X=S(O)_2$, $R_1=4$ -chlorobenzyl, $R_2=-CH_2OH$, $R_3=H$, $R_4=CH_3$, $R_7=H$ and $E=4$ -amidinophenyl), AY ($X=SO_2$, $R_1=3$ -chlorobenzyl, $R_2=-CH_2OH$, $R_3=H$, $R_4=CH_3$, $R_7=H$ and $E=4$ -amidinophenyl) and AZ ($X=SO_2$, $R_1=2$ -fluorobenzyl, $R_2=-CH_2OH$, $R_3=H$, $R_4=CH_3$, $R_7=H$ and $E=4$ -amidinophenyl).

2. Preparation of Preferred Compounds

Figures 1 to 5 depict synthetic schemes for synthesis of intermediates which may be used in preparation of certain compounds of the present invention.

Figure 1 depicts solution phase synthesis of intermediates useful in the preparation of compounds of the present invention. See Examples 60 to 62. See also Examples 95 to 97.

Examples 63 to 66, 67 to 70 and 71 to 73 describe solution phase syntheses of intermediates useful in the synthesis of compounds of the present invention.

Figure 2 depicts an alternate synthetic route to prepare an intermediate useful in the preparation of compounds of the present invention using solution phase synthesis. See also Examples 74 to 76.

Figure 6 depicts a reaction scheme for the preparation of a compound of the present invention having an esterified hydroxyl at P3.

Figure 7 depicts a reaction scheme for the preparation of a compound of the present invention having a 4-guanidinophenyl at P1.

Figure 8 depicts a reaction scheme for the preparation of a compound of the present invention having a 3-guanidinophenyl at P1.

Figure 9 depicts a reaction scheme for the preparation of a compound of the present invention having 2-guanidinothiophenyl at P1.

Figure 11 depicts a reaction scheme for the preparation of a compound of the present invention having a 3-amidinopyridyl at P1.

10 Figure 12 depicts a reaction scheme for the synthesis of a compound of the present invention having 4-amidinophenyl at P1.

15 Figure 14 depicts a reaction scheme for the synthesis of a compound of the present invention having a bicyclic heterocyclic group at P1.

16 Figure 15 depicts a reaction scheme for the synthesis of a compound of the present invention having an amino-imidazolyl group at P1.

20 Figure 16 depicts a reaction scheme for a compound of the present invention having a 2-chloro-4-guanidinophenyl group at P1.

25 Figure 17 depicts a reaction scheme for the synthesis for a compound of the present invention having a guanidino-pyridyl group at P1.

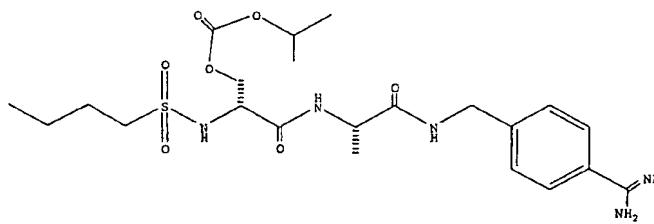
26 Preferred means of chemically coupling (as for example, amide bond function) include formation of a peptide bond by using conventional coupling reagents known in the art. See Bodanszky, N. Peptide Chemistry, pp. 55-73, Springer-Verlag, New York (1988) and references cited therein. The chemical coupling may be either by means of one-step or two-step coupling. In one-step coupling, the two coupling partners are coupled directly. Preferred coupling reagents for one-

step coupling of the coupling partners include DCC with HOBT, EDC with HOBT, EDC with HOAt, HBTU or TBTU. In two-step coupling, an activated ester or anhydride of the C-terminal carboxy group of one coupling partner is formed 5 prior to its coupling to the other coupling partner.

For preparation of certain compounds having hydrogenation-sensitive substituent groups, it is preferred to avoid the use of hydrogen gas with palladium on carbon. Another preferred method for preparing compounds of the 10 present invention containing hydrogenation sensitive groups such as alkenyl or aryl moieties substituted with halogen, cyano, nitro, or $-S-Z_1$, is to use boron tris(trifluoroacetate), $B(OCOCF_3)_3$, to cleave the N^g -nitro of the arginine group. The reagent is prepared by the reaction 15 of BBr_3 and CF_3COOH in dichloromethane at $0^\circ C$. The reagent is also commercially available. Generally, the N^g -nitro compound is treated with boron tris(trifluoroacetate) in trifluoroacetic acid at $0^\circ C$: See, e.g., Fieser, M. and Fieser, L. F., Reagents for Organic Synthesis, p. 46, John 20 Wiley & Sons, New York (1974); Pless, J., and Bauer, W. Angew. Chem., Internat. Ed., 12, 147 (1973).

In addition, another preferred reagent for selective nitro group cleavage is titanium trichloride. This reagent is commercially available. The N^g nitro compound is treated 25 with titanium trichloride in aqueous methanol containing an ammonium acetate buffer followed by exposure of the reaction mixture to air or dimethyl sulfoxide. See, e.g., Freidinger, R.M., Hirschmann, R., and Veber, D.F., J. Org. Chem., 43, 4800 (1978).

30 Figure 6 depicts a reaction scheme for the synthesis of a compound of the present invention where R_2 is $-CH_2OA_1$ and A_1 is $-C(=O)R_6$:



An intermediate such as 6-1 (the compound of Example 9) is reacted with an acid chloride R_6COCl in the presence of a base such as pyridine. Compounds where R_2 is $-(CH_2)_2OA_1$ or $-CH(R_5)OA_1$ where A_1 is $-C(=O)R_6$ may conveniently be prepared by reacting an appropriate intermediate corresponding to 6-1 with the appropriate acid chloride derivative, R_6COCl , preferably in the presence of a base such as pyridine.

Compounds where R_2 is $-(CH_2)_2OA_1$ or $-CH(R_5)OA_1$ wherein A_1 is $-C(=O)OR_6$ may be conveniently prepared by treating a corresponding compound where R_2 is $-(CH_2)_2OH$ or $-CH(R_5)OH$ with the appropriate chloroformate derivative. In the preparation of compounds having an amidino or guanidino group at P₁, it may be preferred to cap the P3 hydroxyl with the carbonate group prior to deprotecting the amidino or guanidino group. Accordingly, it is preferred to treat the corresponding intermediate with the chloroformate derivative. (See, e.g., Example 8). The product is then hydrogenated and optionally treated under hydrolysis conditions to yield the product. (See, e.g., Examples 16, 21, 28, 35 and 40.)

3. Selection of Preferred Compounds

According to one aspect of the present invention, preferred compounds of the present invention are selected for their potency and selectivity toward inhibition of serine proteases, especially urokinase. Such evaluations are routinely performed *in vitro*, following procedures such

as those set forth in Example A. As described therein, and as generally known, a target serine protease and its substrate are combined under assay conditions permitting reaction of the protease with its substrate. The assay is 5 performed in the absence of test compound, and in the presence of increasing concentrations of the test compound. The concentration of test compound at which 50% of the serine protease activity is inhibited by the test compound is the IC₅₀ value (Inhibitory Concentration) or EC₅₀ 10 (Effective Concentration) value for that compound. Within a series or group of test compounds, those having lower IC₅₀ or EC₅₀ values are considered more potent inhibitors of the serine protease than those compounds having higher IC₅₀ or EC₅₀ values. The IC₅₀ measurement is often used for more 15 simplistic assays, whereas the EC₅₀ is often used for more complicated assays, such as those employing cells. K_i is calculated from the IC₅₀.

Preferred compounds according to this aspect of the present invention have a K_i value of 100nM or less as 20 measured in an *in vitro* assay for inhibition of urokinase activity. Especially preferred compounds have a K_i value of less than 30nM.

The test compounds also are evaluated for selectivity toward a serine protease. As described in the Examples, and 25 as generally known, a test compound is assayed for its potency toward a panel of serine proteases and other enzymes and an IC₅₀ value or EC₅₀ value is determined for each test compound in each assay system. A compound that demonstrates a low IC₅₀ value or EC₅₀ value or corresponding low K_i value 30 for the target enzyme, e.g., urokinase, and a higher IC₅₀ value or EC₅₀ value for other enzymes within the test panel (e.g., tissue plasminogen activator, thrombin, Factor Xa),

is considered to be selective toward the target enzyme. Generally, a compound is deemed selective if its IC₅₀ value or EC₅₀ value (or K_i value) in the target enzyme assay is at least one order of magnitude less than the next smallest IC₅₀ 5 value or EC₅₀ value measured in the selectivity panel of enzymes.

Preferred compounds of the present invention have a K_i value of 100nM or less as measured in an *in vitro* assay for inhibition of urokinase activity. Especially preferred 10 compounds have a K_i value in the *in vitro* urokinase inhibition assay that is at least one order of magnitude smaller than the IC₅₀ value measured in the *in vitro* tPA inhibition assay. Compounds having a selectivity ratio of IC₅₀ tPA assay: K_i urokinase assay of greater than 100 are 15 especially preferred.

Compounds of the present invention also are evaluated for their activity *in vivo*. The type of assay chosen for evaluation of test compounds will depend on the pathological condition to be treated or prevented by use of the compound, 20 as well as the route of administration to be evaluated for the test compound.

For instance, to evaluate the activity of a compound of the present invention to reduce tumor growth through inhibition of urokinase, the procedures described by Jankun 25 et al. [Canc. Res. 57:559-563, 1997] to evaluate PAI-1 can be employed. Briefly, the ATCC cell lines DU145, which expresses a high level of uPA, and LnCaP, which does not express uPA, are injected into SCID mice. After tumors are established, the mice are given test compound according to a 30 dosing regime determined from the compound's *in vitro* characteristics. The Jankun et al. compound was administered in water. Tumor volume measurements are taken

twice a week for about five weeks. A compound is deemed active if an animal to which the compound was administered exhibited decreased tumor volume, as compared to animals receiving appropriate control compounds. Furthermore, a 5 comparison of a compound's effect in animals injected with DU145 cells versus LnCaP cells can indicate whether the compound's effect was due to inhibition of urokinase or otherwise.

Another *in vivo* experimental model designed to evaluate 10 the effect of p-aminobenzamidine, a purported urokinase inhibitory compound, on reducing tumor volume is described by Billström *et al.* [Int. J. Cancer 61:542-547, 1995].

To evaluate the ability of a compound of the present invention to reduce the occurrence of, or inhibit, 15 metastasis, the procedures described by Kobayashi *et al.* [Int. J. Canc. 57:727-733d, 1994] can be employed. Briefly, a murine xenograft selected for high lung colonization potential is injected into C57B1/6 mice i.v. (experimental metastasis) or s.c. into the abdominal wall (spontaneous 20 metastasis). Various concentrations of the compound to be tested can be admixed with the tumor cells in Matrigel prior to injection. Daily i.p. injections of the test compound are made either on days 1-6 or days 7-13 after tumor inoculation. The animals are killed about three or four 25 weeks after tumor inoculation, and the lung tumor colonies are counted. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing and route of administration.

The activity of the compounds of the present invention 30 toward decreasing tumor volume and metastasis can be evaluated in the model described by Rabbani *et al.* [Int. J. Cancer 63:840-845, 1995] to evaluate their inhibitor.

There, Mat LyLu tumor cells over-expressing uPA were injected into the flank of Copenhagen rats. The animals were implanted with osmotic minipumps to continuously administer various doses of test compound for up to three weeks. The tumor mass and volume of experimental and control animals were evaluated during the experiment, as were metastatic growths. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing, and route of administration. Some of these authors described a related protocol in Xing et al. [Canc. Res. 57:3585-3593, 1997].

To evaluate the inhibitory activity of a compound of the present invention toward neovascularization, a rabbit cornea neovascularization model can be employed. Avery et al. [Arch. Ophthalmol. 108:1474-1475, 1990] describe anesthetizing New Zealand albino rabbits and then making a central corneal incision and forming a radial corneal pocket. A slow release prostaglandin pellet was placed in the pocket to induce neovascularization. Test compound was administered i.p. for five days, at which time the animals were killed. The effect of the test compound is evaluated by review of periodic photographs taken of the limbus, which can be used to calculate the area of neovascular response and, therefore, limbal neovascularization. A decreased area of neovascularization as compared with appropriate controls indicates the test compound was effective at decreasing or inhibiting neovascularization.

An angiogenesis model used to evaluate the effect of a test compound in preventing angiogenesis is described by Min et al. [Canc. Res. 56:2428-2433, 1996]. C57BL6 mice receive subcutaneous injections of a Matrigel mixture containing bFGF, as the angiogenesis-inducing agent, with and without

test compound. After five days, the animals are killed and the Matrigel plugs, in which neovascularization can be visualized, are photographed. An experimental animal receiving Matrigel and an effective dose of test compound 5 will exhibit less vascularization than a control animal or an experimental animal receiving a less- or non-effective dose of compound.

An *in vivo* system designed to test compounds for their ability to limit the spread of primary tumors is described 10 by Crowley *et al.* [Proc. Natl. Acad. Sci. 90:5021-5025, 1993]. Nude mice are injected with tumor cells (PC3) engineered to express CAT (chloramphenicol acetyltransferase). The cells secrete large amounts of uPA and exhibit saturating amounts of uPA activity bound to uPAR 15 on the cell surface. Compounds to be tested for their ability to decrease tumor size and/or metastases are administered to the animals, and subsequent measurements of tumor size and/or metastatic growths are made. In addition, the level of CAT detected in various organs provides an 20 indication of the ability of the test compound to inhibit metastasis; detection of less CAT in tissues of a treated animal versus a control animal indicates less CAT-expressing cells migrated to that tissue.

In vivo experimental models designed to evaluate the 25 urokinase inhibitory potential of a test compound, using a tumor cell line F3II, said to be highly invasive, are described by Alonso *et al.* [Breast Canc. Res. Treat. 40:209-223, 1996]. This group describes *in vivo* studies for toxicity determination, tumor growth, invasiveness, 30 spontaneous metastasis, experimental lung metastasis, and an angiogenesis assay.

The CAM model (chick embryo chorioallantoic membrane model), first described by L. Ossowski in 1998 [J. Cell Biol. 107:2437-2445, 1988], provides another method for evaluating the urokinase inhibitory activity of a test compound. In the CAM model, invasion of tumor cells through the chorioallantoic membrane is dependent upon the presence of catalytically active uPA. Contacting CAM with tumor cells in the presence of a urokinase inhibitory agent results in less or no invasion of the tumor cells through the membrane. Thus, the CAM assay is performed with CAM and tumor cells in the presence and absence of various concentrations of test compound. The invasiveness of tumor cells is measured under such conditions to provide an indication of the compound's urokinase inhibitory activity. A compound having urokinase inhibitory activity correlates with less tumor invasion.

The CAM model is also used in a standard assay of angiogenesis (i.e., effect on formation of new blood vessels (Brooks, P.C.; Montgomery, A.M.P.; and Cheresh, D.A., Methods in Molecular Biology 129: 257-269 (1999)). According to this model, a filter disc containing an angiogenesis inducer, such as basic fibroblast growth factor (bFGF) is placed onto the CAM. Diffusion of the cytokine into the CAM induces local angiogenesis, which may be measured in several ways such as by counting the number of blood vessel branch points within the CAM directly below the filter disc. The ability of compounds of the present invention to inhibit cytokine-induced angiogenesis can be tested using this model. A test compound can either be added to the filter disc that contains the angiogenesis inducer, be placed directly on the membrane or be administered systemically. The extent of new blood vessel

formation in the presence and/or absence of test compound can be compared using this model. The formation of fewer new blood vessels in the presence of a test compound would be indicative of anti-angiogenesis activity. Since certain 5 of the compounds of the present invention are active as inhibitors of urokinase, anti-angiogenesis activity for such compounds may suggest that urokinase plays a significant role in angiogenesis.

4. Pharmaceutical Compositions

10 In another aspect, the present invention encompasses pharmaceutical compositions prepared for storage or administration which comprise a therapeutically effective amount of a compound of the present invention in a pharmaceutically acceptable carrier.

15 The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to 20 determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled 25 in the medical arts will recognize.

The therapeutically effective amount of the compound of the present invention can range broadly depending upon the desired affects and the therapeutic indication. Typically, dosages will be between about 0.01 mg/kg and 100 mg/kg body

weight, preferably between about 0.01 and 10 mg/kg, body weight.

Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are 5 described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the 10 pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id.

The pharmaceutical compositions of the present 15 invention may be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions and suspensions for injectable administration; and the like. The dose and method of administration can be tailored to achieve optimal 20 efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

When administration is to be parenteral, such as 25 intravenous on a daily basis, injectable pharmaceutical compositions can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, 30 sodium glutamate, or the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting

agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (e.g., liposomes) may be utilized.

5. Utility

5 The compounds of the present invention having urokinase inhibitory activity and/or activity in reducing or inhibiting blood vessel formation, including angiogenesis and neovascularization, may be used both *in vitro* and *in vivo* for a number of applications, some of which are
10 described herein below.

The compounds of the present invention are active as inhibitors of urokinase and specifically bind urokinase. Accordingly those compounds that contain sites suitable for linking to a solid/gel support may be used *in vitro* for
15 affinity chromatography to purify urokinase from a sample or to remove urokinase from a sample using conventional affinity chromatography procedures. These compounds are attached or coupled to an affinity chromatography either directly or through a suitable linker support using
20 conventional methods. See, e.g. Current Protocols in Protein Science, John Wiley & Sons (J.E. Coligan et al., eds, 1997) and Protein Purification Protocols, Humana Press (S. Doonan, ed., 1966) and references therein.

The compounds of the present invention having urokinase
25 inhibitory activity are useful in *in vitro* assays to measure tPA activity in a sample. In assays which measure the total plasminogen activation activity in a blood sample, a compound of the present invention having urokinase inhibiting activity will knock out that portion of
30 plasminogen activation attributable to uPA, which will allow for calculation of the portion of the total plasminogen

activation due to tPA activity as well as that due to uPA activity. Use of such assays to monitor tPA activity would allow better dosage control in patients receiving tPA. These assays could also be used to monitor uPA activity 5 levels in tissue samples, such as from biopsy or to monitor uPA/tPA activities for any clinical situation where measurement of plasminogen activation activity is of assistance. These assays may also be used to monitor plasminogen activator activity where a patient has been 10 treated with a non-endogenous compound having plasminogen activator activity, such as streptokinase and staphlyokinase.

The compounds of the present invention are useful *in vivo* for treatment of pathologic conditions which would be 15 ameliorated by decreased urokinase activity. For example these compounds will inhibit the activation of metalloproteases by the uPA-plasmin cascade in synovial fluid and thus, may be used in treatment of arthritis.

It is believed these compounds will be useful in 20 decreasing or inhibiting metastasis, neovascularization, and degradation of the extracellular matrix in tumors and other neoplasms. These compounds will be useful as therapeutic agents in treating conditions characterized by pathological neovasculararation such as retinal disease, retinopathies and 25 other conditions, including those described hereinabove in the Background and Introduction to the Invention.

Another use for the compounds of the present invention having urokinase inhibitory activity is as an antidote if too much exogenous urokinase has been given to a patient for 30 therewith purposes, such as for dissolving a blood clot.

The compounds of the present invention may be used in treating conditions characterized by inflammation due to

their anti-inflammatory effects from inhibition of urokinase, thereby interfering with mediators of cell adhesion or migration. Such anti-inflammatory applications include treatment of stroke and complications of organ transplants.

The present invention includes methods for preventing or treating a condition in a mammal suspected of having a condition which will be attenuated by inhibition of urokinase activity comprising administering to said mammal a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

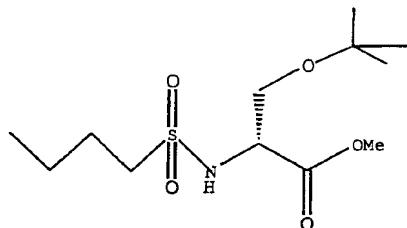
The compounds or pharmaceutical compositions of the present invention are administered *in vivo*, ordinarily in a mammal, preferably in a human. In employing them *in vivo*, the compounds or pharmaceutical compositions can be administered to a mammal in a variety of ways, including orally, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms. Administration is preferably oral, such as by tablets capsules or elixirs taken on a daily basis.

In practicing the methods of the present invention, the compounds or pharmaceutical compositions of the present invention are administered alone or in combination with one another, or in combination with other therapeutic or *in vivo* diagnostic agents.

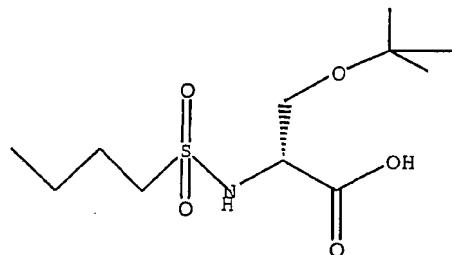
As is apparent to one skilled in the medical art, a "therapeutically effective amount" of the compounds or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, the particular mode of administration and the desired affects and the

therapeutic indication. Because these factors and their relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the 5 desired result of inhibiting uPA activity, will be within the ambit of one skilled in these arts. Typically, administration of the compounds or pharmaceutical composition of the present invention is commenced at lower dosage levels, with dosage levels being increased until the 10 desired effect of inhibiting uPA activity to the desired extent is achieved, which would define a therapeutically effective amount. For the compounds of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.01 mg/kg and 100 mg/kg body 15 weight, preferably between about 0.01 mg/kg and 10 mg/kg, body weight.

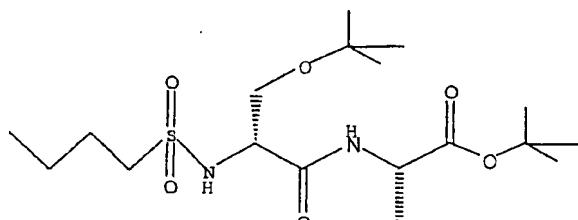
To assist in understanding, the present invention will now be further illustrated by the following examples. These examples as they relate to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

ExamplesA. Synthesis of Certain Compounds of the Present InventionExample 1Preparation of n-butylsulfonyl-D-serine(tert-butylether)-5-methyl ester (1)

A solution of HCl·H-dSer(tBu)-OMe (2 g, 9.44 mmol) and n-butylsulfonyl chloride (1.1 ml, 8.50 mmol) in tetrahydrofuran (38 ml) was stirred for ten minutes at room temperature. Diisopropylethylamine (5.75 ml, 33.07 mmol) was then added and the cloudy yellow solution was stirred over night at ambient temperature. The reaction mixture was then diluted with ethylacetate (200 ml) and washed with 1N HCl, followed by brine (20 ml each). After drying over anhydrous sodium sulfate, the solvents were removed under vacuum. The flaky yellow solid (1.56 g, 62%) was judged pure by tlc (5% ethylacetate in hexanes).

Example 2Preparation of n-butylsulfonyl-D-serine(tert-butylether) (2)

To a solution of compound 1 (1.46 g, 4.94 mmol) in
 5 dioxane (32.95 ml), was added dropwise 2.0 N LiOH (5.44 ml,
 10.87 mmol). The cloudy yellow solution was allowed to stir
 at ambient temperature overnight. When no starting material
 was observed by tlc (5% ethylacetate/hexanes), the excess
 dioxane was removed *in vacuo*. The reaction mixture was
 10 diluted with a 1:1 mixture of water and methanol and passed
 through a pre-washed DOWEX (50 × 8-400) ion exchange resin
 (30 ml). The resin was rinsed thoroughly with methanol and
 water. The combined filtrates were concentrated under
 reduced pressure to afford 1.44 g of the title compound in
 15 quantitative yield as a cream solid.

Example 3Preparation of n-butylsulfonyl-D-serine(tert-butylether)-alanine tert-butylester (3)

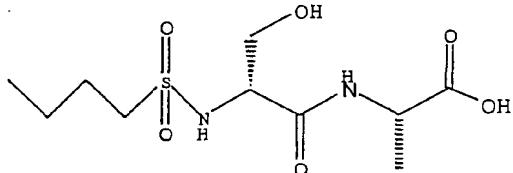
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A solution of the compound of Example 2 (0.50 g, 1.79
 mmol), alanine tert-butylester hydrochloride salt (0.65 g,

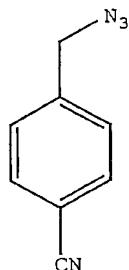
3.58 mmol), EDC (0.68 g, 3.57 mmol), N-hydroxybenzotriazole (0.27 g, 1.79 mmol) and diisopropylethylamine (1.56 ml, 8.94 mmol) was stirred in acetonitrile (18 ml) at ambient temperature. After 18 hours, the solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (50 ml) and 1N HCl (10 ml). The ethylacetate layer was washed with 1N HCl (10 ml), saturated sodium bicarbonate (2 x 15 ml) and brine (15 ml), then dried with sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography eluting with 50% ethyl acetate/ hexanes, yielding 429 mg (59%) product. The product was a single peak by reverse phase (C18) HPLC ($t_R = 9$ minutes at 0.1% trifluoroacetic acid in 5-90% aqueous acetonitrile over 20 minutes).

Example 4

Preparation of n-butylsulfonyl-D-serine-alanine (4)



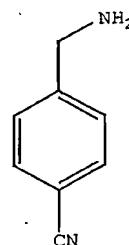
To a solution of the compound of Example 3 (0.42 g, 20 1.02 mmol) in dichloromethane (4.2 ml) was added trifluoroacetic acid (4.2 ml). The reaction mixture was stirred at ambient temperature for 1 hour. The reaction mixture was diluted with 50 ml n-heptane and concentrated *in vacuo*. The residue was resuspended in 10 ml acetonitrile 25 and 50 ml n-heptane and concentrated *in vacuo* to yield 410 mg product.

Example 5Preparation of α -azido-4-cyanotoluene (5)

Sodium azide (Aldrich, 3.5 g, 54 mmol) was added to a
5 solution of α -Bromotoluonitrile (Aldrich, 10 g, 51 mmol) in
DMF (100 ml), and the resulting mixture was stirred at
ambient temperature for 5 hours. The reaction mixture was
then diluted with water (350 ml) and extracted with ether (2
x 100 ml). The combined organic layers were washed with
10 brine and dried ($MgSO_4$). Removal of solvent led to the title
compound (8 g, 96%). 1H NMR ($CDCl_3$): δ 4.42 (s, 2H), 7.41
(d, 2H, J = 8.1 Hz), 7.65 (d, 2H, J = 8.1 Hz).

Example 6Preparation of 4-(aminomethyl)benzylnitrile (6)

15

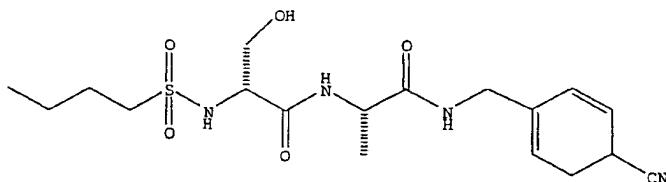


The catalyst of 10% Pd-on-C (Aldrich, 800 mg) was added
to a solution of α -azido-4-cyanotoluene (compound 5, 8 g, 51
mmol) in EtOAc (150 ml). The reaction mixture was

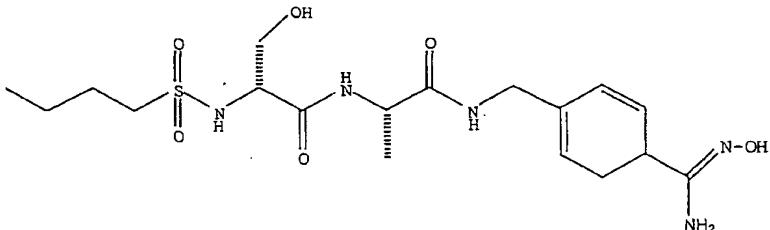
hydrogenated (H_2 , 45 psi) in a Parr apparatus for 11 hours. Catalyst was filtered and the solvent was removed under vacuum to give the title compound (6.3 g, 93%). 1H NMR ($CDCl_3$): δ 3.85 (s, 2H), 7.45 (d, 2H, J = 8.1), 7.60 (d, 2H, J = 8.1 Hz), 7.78 (s, 2H, NH₂).

Example 7

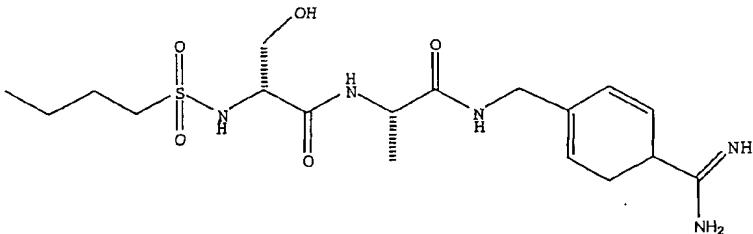
Preparation of n-butylsulfonyl-D-serine-alanine-4-cyanobenzylamide (7)



10 A solution of compound 4 (150 mg, 0.51 mmol), 4-(aminomethyl)benzyl nitrile (compound 6, 171 mg, 1.02 mmol), EDC (195 mg, 1.02 mmol), and N-hydroxybenzotriazole (78 mg, 0.51 mmol) in acetonitrile (5.1 ml) was stirred at ambient temperature for 10 minutes. 2,4,6-Collidine (0.34 ml, 2.54 mmol) was then added and the reaction mixture was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethyl acetate (100 ml) and 0.5M HCl (10 ml). The ethyl acetate layer was washed with water followed by 20 0.5M HCl (10 ml), saturated sodium bicarbonate (2 × 10 ml) and brine (15 ml), then dried with sodium sulfate. The solvent was removed under reduced pressure to yield 237 mg product. The product eluted at 9.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

Example 8Preparation of n-butylsulfonyl-D-serine-alanine-4-hydroxymidinobenzylamide (8)

5 To a solution of the product in Example 7 (117 mg, 0.285 mmol) in 1.14 ml methanol was added hydroxylamine hydrochloride (33.7 mg, 0.485 mmol), followed by N-methylmorpholine (53 µl, 0.485 mmol). The reaction mixture was stirred overnight at ambient temperature and then at 50°C
10 for six hours. The reaction mixture was concentrated *in vacuo*. The crude product was taken to the next step (Example 9) without further purification. The product eluted at 6.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20
15 minutes.

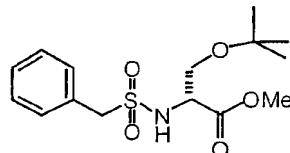
Example 9Preparation of n-butylsulfonyl-D-serine-alanine-4-amidinobenzylamide (9)

To the product of Example 8 (126 mg, 0.285 mmol) in acetic acid (2.85 ml) and water (0.28 ml) was added 185 mg activated zinc dust. The reaction mixture was stirred overnight at room temperature. The zinc dust was filtered 5 using a glass funnel and the filtrate was purified by preparative HPLC. The fractions containing the product eluted in a 5-20% aqueous acetonitrile containing 0.1% TFA and were pooled and lyophilized yielding 35 mg of the title compound as a white powder. The product eluted at 6.0 10 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes.

Example 10

Preparation of benzylsulfonyl-D-serine(tert-butylether)-methyl ester (10)

15

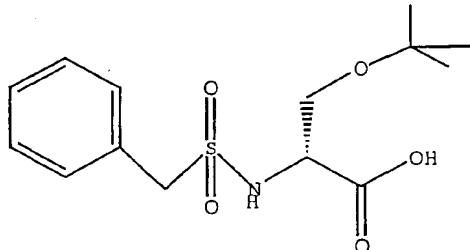


A solution of HCl·H-dSer(tBu)-OMe (1 g, 4.72 mmol) and phenethylsulfonyl chloride (1.45 g, 7.08 mmol) in acetonitrile (19 ml) was stirred for ten minutes at room temperature. Diisopropylethylamine (1.53 ml, 11.81 mmol) 20 was then added and the clear yellow solution was stirred for 18 hours at ambient temperature. The reaction mixture was then diluted with ethylacetate (100 ml) and washed with 1N HCl, followed by brine (10 ml each). After drying over anhydrous sodium sulfate, the solvents were removed *in vacuo*. The crude product was purified by flash column chromatography eluting with dichloromethane, followed by a gradient consisting of 1 to 5% ethylacetate in 25 dichloromethane, yielding 840 mg (52%) product. Tlc of the

final product in 5% ethylacetate in dichloromethane gave one spot with an *R_f* of 0.52.

Example 11

Preparation of benzylsulfonyl-D-serine(tert-butylether) (11)

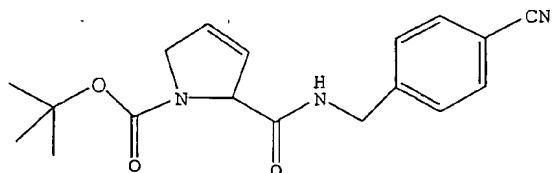


5

To a solution of the compound of Example 10 (1.0 g, 3.03 mmol) in dioxane (20 ml), was added dropwise 2.0N LiOH (3.33 ml, 6.67 mmol). The solution was allowed to stir at ambient temperature overnight. The excess dioxane was removed *in vacuo*. The reaction mixture was diluted with a 1:1 mixture of water and methanol and passed through a pre-washed DOWEX (50 × 8-400) ion exchange resin (30 ml). The resin was rinsed thoroughly with methanol and water. The combined filtrates were concentrated under reduced pressure to afford 1.10 g of the title compound as a yellow glue.

Example 12

Preparation of tert-butyloxycarbonyl-3,4-dehydroproline p-cyanobenzylamide (12)

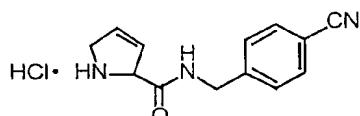


A solution of tert-butyloxycarbonyl-3,4-dehydroproline (0.4 g, 1.88 mmol), 4-(aminomethyl)benzyl nitrile (compound

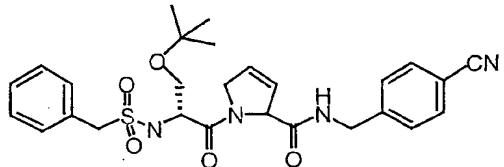
6, 0.47 g, 2.82 mmol), EDC (0.54 g, 2.82 mmol), N-hydroxybenzotriazole (0.29 g, 1.88 mmol), and diisopropylethylamine (1.64 ml, 9.39 mmol) in acetonitrile (7.5 ml) was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (25 ml) and 0.5M HCl (5 ml). The ethylacetate layer was washed with water followed by 0.5M HCl (5 ml), saturated sodium bicarbonate (2 × 5 ml) and brine (10 ml), then dried with sodium sulfate. The solvent was removed under reduced pressure and the crude was purified by flash column chromatography eluting with 4/1 ethylacetate/hexane to yield 561 mg pure product (91.3%). The product eluted at 10.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

Example 13

Preparation of 3,4-dehydropyroline-4-cyano benzylamide hydrochloride salt (13)



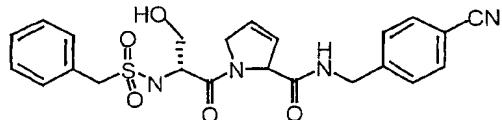
To a solution of the compound of Example 12 (0.47 g, 1.44 mmol) in ethylacetate (5.7 ml) was added 5M anhydrous HCl in ethylacetate (1.44 ml) and the reaction was stirred at ambient temperature overnight. The reaction mixture was concentrated *in vacuo* to yield 363 mg (95%) of a white solid.

Example 14Preparation of benzylsulfonyl-D-serine(tert-butylether)-proline(dehydro)-4-cyanobenzylamide (14)

5 A solution of the compound of Example 11 (0.10 g, 0.32 mmol), the compound of example 13 (0.092 g, 0.34 mmol), EDC (0.091 g, 0.48 mmol), and N-hydroxybenzotriazole (0.053 g, 0.35 mmol) was stirred in acetonitrile (1.2 ml) for 10 minutes. 2,4,6-Collidine (0.209 ml, 1.58 mmol) was then added and the reaction mixture was stirred over night at ambient temperature. The solvent was removed under reduced pressure. The resulting residue was resuspended in ethylacetate (50 ml) and 1N HCl (10 ml). The ethylacetate layer was washed with 1N HCl (10 ml), saturated sodium bicarbonate (2 x 15 ml) and brine (15 ml), then dried with sodium sulfate to a yellow syrup (160 mg, 94%). The product was a single peak by reverse phase (C18) HPLC ($t_R = 11$ minutes at 0.1% trifluoroacetic acid in 5-90% aqueous acetonitrile over 20 minutes).

10

15

Example 15Preparation of benzylsulfonyl-D-serine-3,4-dehydroproline-4-cyanobenzylamide (15)

20 To a solution of the compound of Example 14 (0.16 g, 0.30 mmol) in dichloromethane (0.6 ml) was added

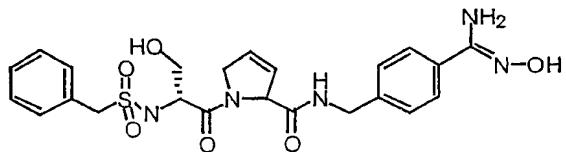
25

trifluoroacetic acid (0.6 ml). The reaction mixture was stirred at ambient temperature for 1 hour. The reaction mixture was diluted with 10 ml n-heptane and concentrated *in vacuo*. The residue was resuspended in 5 ml acetonitrile and 5 10 ml n-heptane and concentrated *in vacuo* to yield 183 mg product.

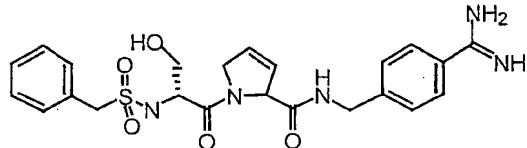
Example 16

Preparation of benzylsulfonyl-D-serine-3,4-dehydroproline-4-hydroxyamidinobenzylamide (16)

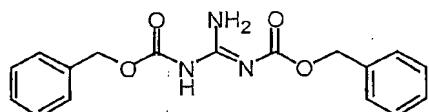
10



To a solution of the product in Example 15 (143 mg, 0.305 mmol) in 1.22 ml methanol was added hydroxylamine hydrochloride (0.036 mg, 0.519 mmol) followed by N-methylmorpholine (57 μ l, 0.519 mmol). The reaction mixture 15 was stirred over night at ambient temperature. Analytical HPLC suggested that the reaction was not complete. Additional hydroxylamine hydrochloride (0.036 mg, 0.519 mmol) and N-methylmorpholine (57 μ l, 0.519 mmol) were added and stirring continued at ambient temperature over night. 20 The reaction mixture was concentrated *in vacuo* and the crude was purified by preparative HPLC. The fractions containing the product eluting in 5-20% aqueous acetonitrile containing 0.1% TFA solution were pooled and lyophilized yielding 16 mg of the title compound as a white powder.

Example 17Preparation of benzylsulfonyl-D-serine-3,4-dehydroproline-p-amidinobenzylamide (17)

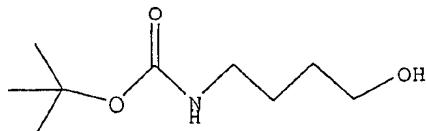
5 To the product of Example 16 (15 mg, 0.030 mmol) in acetic acid (0.30 ml) and water (0.03 ml) was added 19 mg activated zinc dust. The reaction mixture was stirred over night at room temperature. The zinc dust was filtered using a glass funnel and the filtrate was purified by preparative HPLC. The fractions containing the product eluted in a 5-10 20% aqueous acetonitrile containing 0.1% TFA, and were pooled and lyophilized yielding 7 mg of the title compound as a white powder. The product eluted at minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% 15 aqueous acetonitrile over 20 minutes.

Example 18Preparation of bis(benzyloxycarbonyl) guanidine (18)

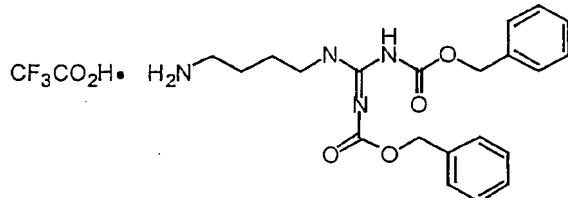
20 The synthesis of the title product was carried out as cited in the literature (*Tetrahedron Letters*, vol. 35, No. 7, pp. 977-980, 1994) and is described below:

A solution of N,N'-bis(benzyloxycarbonyl)-S-methylisothiourea (1 g, 2.79 mmol) in 7N anhydrous ammonia in methanol (5.5 ml) was stirred overnight at ambient 25 temperature. The reaction mixture was concentrated and the

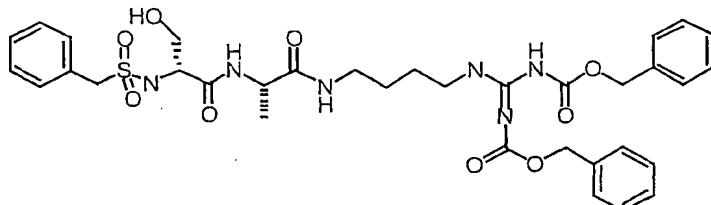
remaining residue was diluted with ethylacetate (10 ml). The organic layer was washed twice with saturated sodium bicarbonate and once with brine (10 ml each). After drying over sodium sulfate, the crude product was subjected to 5 flash column chromatography eluting with 3/2 ethylacetate/hexanes to yield 0.87 g of a white solid. The product was then recrystallized in 1:1 ethylacetate: hexane solvent system to yield 337 mg (37%) pure product (mp= 151 °C).

10 Example 19Preparation of tert-butyloxycarbonyl-4-amino-1-butanol (19)

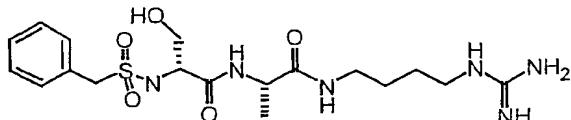
To a solution of 4-amino-1-butanol (1 g, 11.22 mmol) in tetrahydrofuran (45 ml) was added Boc-anhydride (2.20 g, 15 10.10 mmol) and triethylamine (2.84 g, 28.04 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the remaining residue was diluted with ethylacetate (250 ml) and 1M HCl (50 ml). The layers were separated and the organic layer 20 was washed with 1N HCl, water and brine (50 ml each). After drying over sodium sulfate 1.9 g (99%) product was obtained as a clear oil.

Example 20Synthesis of g-N,N'-bis(benzyloxycarbonyl)agmatine trifluoroacetate salt (20)

5 To a solution of compound 18 (the product of Example
18) (0.2 g, 0.61 mmol) and triphenylphosphine (0.12 g, 0.46
mmol) in dry toluene (6.6 ml) under nitrogen was added via
syringe compound 19 (the product of Example 19). The
mixture was cooled to 0°C, and diethylazodicarboxylate (0.080
10 g, 0.46 mmol) was added dropwise over 15 minutes. The
reaction mixture was stirred at room temperature over night
at which time tlc in 3/2 ethylacetate/hexane confirmed the
completion of the reaction. Five drops of water were added
and the solvent was evaporated *in vacuo*. The crude was
15 purified by flash column chromatography eluting with 9/1
hexanes/ethylacetate, followed by 3/2 hexanes/ethylacetate
to yield 83 mg (74%) pure product. The product was then
treated with dichloromethane and trifluoroacetic acid (1 ml
each) for one hour at ambient temperature. Removal of the
20 solvents *in vacuo* afforded 80 mg product 20.

Example 21Preparation of n-butylsulfonyl-D-serine-alanine-agmatine-g-N,N-bis(benzyloxycarbonyl) (21)

5 A solution of the compound of Example 4 (0.05 g, 0.17 mmol), and the compound of Example 20 (0.065 g, 0.17 mmol), EDC (0.065 g, 0.34 mmol), and hydroxybenzotriazole (0.026 g, 0.17 mmol) was stirred in acetonitrile (0.67 ml) for 10 minutes. 2,4,6-Collidine (0.11 ml, 0.84 mmol) was then 10 added and the reaction mixture was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate and 1N HCl (5 ml each). The ethylacetate layer was washed with 1N HCl (5 ml), saturated sodium bicarbonate 15 (2 x 5 ml) and brine (5 ml), then dried with sodium sulfate to a solid (114 mg, 94%).

Example 22Preparation of n-butylsulfonyl-D-serine-alanine-agmatine (22)

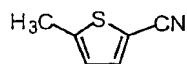
20

The compound of Example 21 (114 mg, 0.17 mmol) was dissolved in methanol (15 ml) and was hydrogenated on a Parr shaker overnight at 40 psi in the presence of 15 mg

palladium on charcoal. The catalyst was filtered off. The reaction mixture was diluted to 35 ml with water and purified by preparative HPLC. The fractions containing the product eluted in 0-25% aqueous acetonitrile containing 0.1%
5 TFA and were pooled and lyophilized yielding 16 mg of the title compound as a white powder.

Example 23

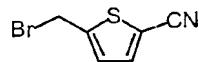
Preparation of 2-cyano-5-methylthiophene (23)



10 A solution of 2-bromo-5-methylthiophene (TCI chemicals, 5 g, 28 mmol) and copper(I) cyanide (Aldrich, 2.53 g, 28 mmol) in DMF (10 ml) was heated at its reflux temperature for 4 hours. After cooling to ambient temperature, ethyl acetate (500 ml) and 10% NaCN aqueous solution (500 ml) was
15 added. After separation, the aqueous layer was extracted with ethyl acetate (300 ml), and the combined organic layers were concentrated to an oil. The oil was further purified by a flash column chromatography (ethyl acetate) to give the title compound (3.03 g, 87%). TLC: Rf 0.30 (1:1 of
20 hexane/ethyl acetate); ^1H NMR (CDCl_3): δ 2.55 (m, 3H), 6.76 (d, 1H, J = 3.6 Hz), 7.42 (d, 1H, J = 3.6 Hz).

Example 24

Preparation of 2-cyano-5-(bromomethyl)thiophene (24)

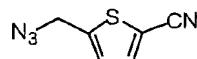


25 A solution of 2-cyano-5-methylthiophene (compound 23, 3.0 g; 24 mmol), N-bromosuccinimide (Aldrich, 4.8 g, 27 mmol), and 2,2'-azobisisobutyronitrile (Aldrich, 0.4 g, 2.4

mmol) in CCl_4 (Aldrich, 60 ml) was heated at its reflux temperature for 5 hours. After cooling to ambient temperature, the solvent was removed under vacuum to give a yellow oil. The oil was purified by a flash column chromatography (1:1 of hexane/ethyl acetate) to give the title compound (4.5 g, 91%). TLC: R_f 0.91 (1:1 of hexane/ethyl acetate); ^1H NMR (CDCl_3): δ 4.66 (s, 2H), 7.10 (d, 1H, J = 3.8 Hz), 7.48 (d, 1H, J = 3.8 Hz).

Example 25

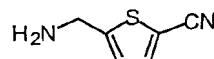
10 Preparation of 2-cyano-5-(azidomethyl)thiophene (25)



A solution of 2-cyano-5-(bromomethyl)thiophene (compound 24, 3.5 g, 17.3 mmol) and sodium azide (Aldrich, 1.7 g, 26 mmol) in DMF (Aldrich, 60 ml) was stirred at ambient temperature for 10 hours. Flash column chromatography (20% ethyl acetate in hexane) resulted the title compound (2.35 g, 83%). TLC: R_f 0.48 (20% of ethyl acetate in hexane); ^1H NMR (CDCl_3): δ 4.56 (s, 2H), 7.01 (d, 1H, J = 3.7 Hz), 7.55 (d, 1H, J = 3.7 Hz).

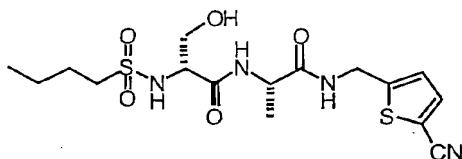
20 Example 26

Preparation of 2-cyano-5-(aminomethyl)thiophene (26)

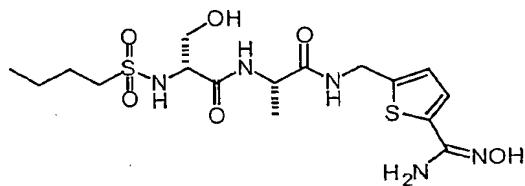


Triphenylphosphine (Aldrich, 5.7 g) was added to a solution of 2-cyano-5-(azidomethyl)thiophene (compound 25, 2.5 g, 10 mmol) in THF (Aldrich, 40 ml) and water (10 ml) at 0°C. The resulting solution was allowed to warm to room temperature and stirred at ambient temperature for 10 hours.

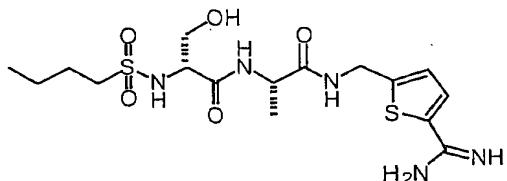
RP HPLC purification led to the title compound (2.3 g, 94%).
MS (electrospray): 139 (M + 1); ^1H NMR (CDCl_3): δ 4.01 (s, 2H), 4.75 (br s, 2H, NH₂), 6.82 (d, 1H, J = 3.5 Hz), 7.08 (d, 1H, J = 3.5 Hz).

5 Example 27Preparation of n-butylsulfonyl-D-serine-alanine-2-cyano-5-(methylamide)thiophene (27)

A solution of the compound of example 4 (4, 860 mg, 2.9 mmol), and the compound of Example 26 (26, 400 mg, 2.9 mmol), EDC (556 mg, 2.9 mmol), N-hydroxybenzotriazole (488 mg, 3.19 mmol) and diisopropylethylamine (1.5 ml, 8.7 mmol) was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (50 ml) and 1N sodium bisulfate (10 ml). The ethylacetate layer was washed with 1N sodium bisulfate (10 ml), saturated sodium bicarbonate (2 x 15 ml) and brine (15 ml), then dried with sodium sulfate to give a yellow oil. The crude product was subjected to flash column chromatography eluting with a 4/5/1 ratio of ethylacetate/hexanes/methanol. The product was a 3:1 mixture of diastereomers by reverse phase (C18) HPLC ($t_{\text{R}} = 8.5$ minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 30 minutes). Low resolution mass spectroscopy confirmed the desired mass ($\text{MH}^+ = 417$).

Example 28Synthesis of n-butylsulfonyl-D-serine-alanine-2-hydroxyamidino-5-(methylamide)thiophene (28)

5 To a solution of the product in Example 27 (220 mg, 0.53 mmol) in 5 ml methanol was added hydroxylamine hydrochloride (184 mg, 2.64 mmol) followed by N-methylmorpholine (290 μ l, 2.64 mmol). The reaction mixture was stirred overnight at ambient temperature. The methanol
10 mixture was concentrated *in vacuo* and the remaining residue was diluted with ethylacetate and washed with saturated sodium bicarbonate and brine (10 ml each). The organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give a yellow oil (120 mg, 50%).
15 The product was a 3:1 mixture of diastereomers by reverse phase (C18) HPLC (t_R = 5 minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes). Low resolution mass spectrum confirmed the desired mass (MH^+ 450.5).

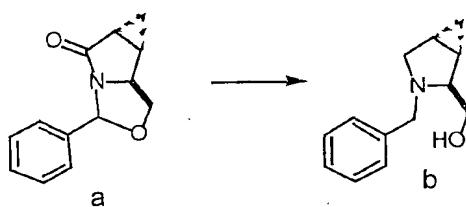
20 Example 29Synthesis of n-butylsulfonyl-D-serine-alanine- 2-amidino-5-(methylamide)thiophene (29)

To the product of Example 28 (60 mg, 0.13 mmol) in acetic acid (1.3 ml) and water (0.13 ml) was added 87 mg activated zinc dust. The reaction mixture was stirred overnight at ambient temperature. The zinc dust was 5 filtered using a glass funnel and the filtrate was purified by preparative HPLC. The fractions containing the product eluted in 0-20% aqueous acetonitrile containing 0.1% TFA. They were pooled and lyophilized yielding 7 mg of the title compound as a white powder. The 3:2 diastereomeric mixture 10 of products eluted at 13 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes. Low resolution mass spectroscopy confirmed the desired mass (MH^+ 434).

Example 30

15 Synthesis of tert-butyloxycarbonyl-3,4-methanoproline (30)
(steps A to E below)

Step A. Synthesis of N-benzyl-3,4-methanoprolinol (b)

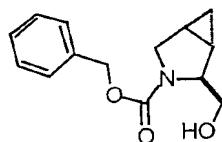


20 A mixture of the benzylidene starting material (a) (J. Org. Chem. 1999, 64(2), 547) (4.6 grams, 21.4 mmol) and lithium aluminum hydride (1.0M in THF, 64 ml, 64 mmol) was heated at reflux for 5 hours. After cooling to 0°C, the remaining aluminum hydride was carefully quenched by the 25 dropwise addition of saturated aqueous sodium sulfate (5 ml) over 15 minutes. The mixture was diluted with ethylacetate

(200 ml) and then filtered through celite. The filtrate was dried with sodium sulfate, filtered and concentrated to give crude N-benzyl aminoalcohol (3.45 grams), which was carried on to the next step without further purification.

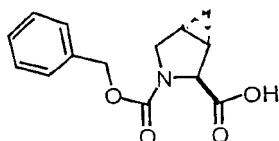
5

Step B. Synthesis of N-benzyloxycarbonyl-3,4-methanoprolinol



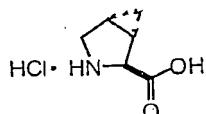
A solution of crude N-benzyl-3,4-methanoprolinol (step 10 A, (b)) (3 grams, 14.76 mmol) in methanol (120 ml) and concentrated HCl (1.5 ml) with 10% Pd/C (300 mg) was hydrogenated at 50 psi for 16 hours. The reaction mixture was filtered to remove the carbon-based catalyst and the filtrate was concentrated. The residue was dissolved in water/dioxane (100 ml) and diisopropylethylamine (3.2 ml) was added. Benzyl chloroformate (2.76 ml, 16.2 mmol) was added and the reaction mixture was stirred overnight. The reaction mixture was concentrated, dissolved in 1M HCl (100 ml) and extracted with ethylacetate (3 x 200 ml). The combined organic layers were dried with magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography using 1:3 ethylacetate/hexanes to give the N-Cbz-3,4-methanoprolinol (2.4 g).

Step C. Synthesis of N-benzyloxycarbonyl-3,4-methanoproline



To a solution of N-benzyloxycarbonyl-3,4-methanoprolinol (2.2 g, 8.90 mmol) in acetone (68 ml),
5 stirring at 0°C, was added Jones reagent (6.6 ml) dropwise over 5 minutes. [Jones Reagent: Prepared from chromium trioxide (13.4 g) and concentrated sulfuric acid (11.5 ml) diluted with water to a total volume of 50 ml.] After
10 stirring at 0°C for 3 hours, isopropanol (11 ml) was added and stirring was continued for an additional 10 minutes. The reaction mixture was diluted with water (400 ml) and extracted with ethylacetate (3 x 500 ml). The combined organic layers were dried over magnesium sulfate, filtered
15 and concentrated to give N-Cbz-3,4-methanoproline (2.25 g, 96%).

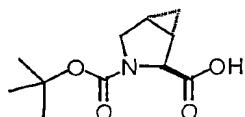
Step D. Synthesis of 3,4-methanoproline hydrochloride salt



20 To a solution of N-benzyloxycarbonyl-3,4-methanoproline (obtained in step C, above) (1.23 g, 4.71 mmol) in ethanol (47 ml) and 0.5M HCl (9.42 ml) was added 10% palladium on carbon catalyst. The reaction mixture was hydrogenated at
25 atmospheric pressure overnight. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to yield 767

mg pure product. The product eluted at 14 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

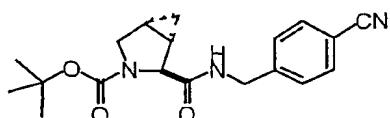
5 Step E. Preparation of tert-butyloxycarbonyl-3,4-methanoproline



To a solution of 3,4-methanoproline hydrochloride salt (from step D above) (1.04 g, 6.38 mmol) in dioxane and water 10 (25 ml each) was added potassium carbonate (1.76 g, 12.76 mmol) and Boc-anhydride (2.78 g, 12.76 mmol). The reaction mixture was stirred overnight at ambient temperature. The dioxane was removed *in vacuo* and the remaining residue was diluted with diethylether. The layers were separated and 15 the aqueous layer was extracted with diethyl ether (1 × 25 ml). The aqueous layer was acidified to pH <3 with 1N hydrochloric acid and extracted with ethylacetate (3 × 25 ml). The organic layer was dried over sodium sulfate, decanted and concentrated under reduced pressure to afford 20 745 mg (50%) product. The product eluted at 12.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

Example 31

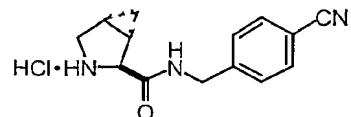
Preparation of tert-butyloxycarbonyl-3,4-methanoproline-4-cyanobenzylamide (31)



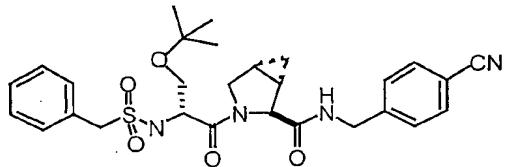
A solution of the compound of Example 30 (step E) (0.70 g, 3.082 mmol), p-cyanobenzylamine hydrochloride salt (0.784 g, 4.62 mmol), EDC (0.88 g, 4.62 mmol), N-hydroxybenzotriazole (0.47 g, 3.082 mmol) and diisopropylethylamine (2.68 ml, 15.41 mmol) was stirred in acetonitrile (12 ml) at ambient temperature. After 18 hours, the solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (150 ml) and washed consecutively with 0.5 M HCl (2 × 15 ml), brine (1 × 15 ml), saturated sodium bicarbonate (2 × 15 ml), and brine (1 × 15 ml). The organic layer was dried over sodium sulfate, decanted and concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with 4/1 ethyl acetate/hexanes, yielding 822 mg (70%) product.

Example 32

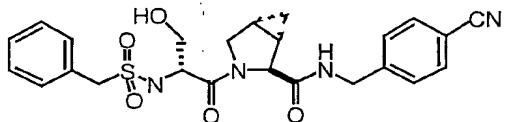
Preparation of 3,4-methanoproline-4-cyanobenzylamide hydrochloride salt (32)



To a solution of the compound of Example 31 (1 g, 2.93 mmol) in ethylacetate (11.7 ml) was added 5M anhydrous HCl in ethylacetate (2.93 ml) and the reaction was stirred at ambient temperature overnight. The reaction mixture was concentrated *in vacuo* to yield 645 mg (79%) of a white solid.

Example 33Preparation of benzylsulfonyl-D-serine(tert-butylether)-3,4-methanoproline-4-cyanobenzylamide (33)

5 A solution of the compound of Example 11 (0.10 g, 0.32 mmol), the compound of Example 32 (0.105 g, 0.38 mmol), EDC (0.073 g, 0.38 mmol), N-hydroxybenzotriazole (0.049 g, 0.32 mmol), and diisopropylethylamine (0.28 ml, 1.59 mmol) was stirred overnight at ambient temperature. The solvent was
 10 removed under reduced pressure and the resulting residue was resuspended in ethylacetate (50 ml) and 1N HCl (10 ml). The ethylacetate layer was washed with 1N HCl (10 ml), saturated sodium bicarbonate (2 x 15 ml) and brine (15 ml), then dried with sodium sulfate. The organic layer was decanted and
 15 concentrated under reduced pressure to yield 171 mg product.

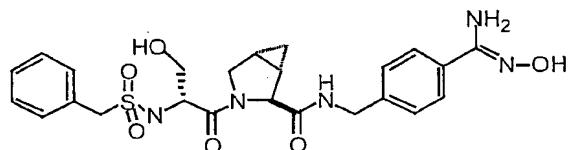
Example 34Preparation of benzylsulfonyl-D-serine-3,4-methanoproline-4-cyanobenzylamide (34)

20 To a solution of the compound of Example 33 (0.17 g, 0.32 mmol) in dichloromethane (4 ml) was added trifluoroacetic acid (4 ml). The reaction mixture was stirred at ambient temperature for 1 hour. The reaction mixture was diluted with 10 ml n-heptane and concentrated *in vacuo*. The residue was resuspended in 5 ml acetonitrile and
 25

10 ml n-heptane and concentrated *in vacuo* to yield 154 mg product.

Example 35

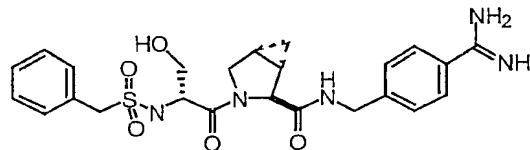
Preparation of benzylsulfonyl-D-serine-3,4-methanoproline-4-hydroxyamidinobenzylamide (35)



To a solution of the product in Example 34 (154 mg, 0.32 mmol) in 1.3 ml methanol was added hydroxylamine hydrochloride (0.11 g, 1.58 mmol) followed by N-methylmorpholine (209 μ l, 1.902 mmol). The reaction mixture was stirred overnight at ambient temperature. The reaction mixture was concentrated *in vacuo*. The crude product was resuspended in 25% acetonitrile in water and purified by preparative HPLC. The fractions containing the product eluting in 0-20% aqueous acetonitrile containing 0.1% TFA solution were pooled and lyophilized yielding 19.5 mg of the title compound as a white powder. Low resolution mass spectroscopy confirmed the desired mass (MH^+ 516).

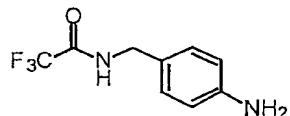
Example 36

Preparation of benzylsulfonyl-D-serine-3,4-methanoproline-p-amidinobenzylamide (36)

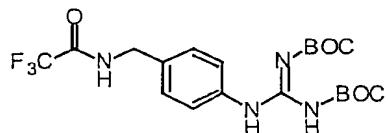


To the product of Example 35 (19.5 mg, 0.041 mmol) in acetic acid (0.41 ml) and water (0.041 ml) was added 27 mg activated zinc dust. The reaction mixture was stirred over

night at room temperature. The zinc dust was filtered using a glass funnel and the filtrate was purified by preparative HPLC. The fractions containing the product eluting in 0-20% aqueous acetonitrile containing 0.1% TFA were pooled and 5 lyophilized yielding 15 mg of the title compound as a white powder. The product eluted at 10.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes. Low resolution mass spectroscopy confirmed the desired mass (MH^+ 500).

10 Example 37Preparation of 4-trifluoracetamidomethylaniline (7-2)

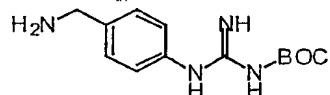
4-Nitrobenzylamine (7-1) (4.0 g, 21 mmol) was added in portions to trifluoroacetic anhydride (15 ml) while the 15 mixture was being cooled on ice. The mixture was allowed to warm to room temperature and stirred overnight. The suspension was poured onto ice (approximately 200 g), and the cloudy suspension was extracted with CH_2Cl_2 (2 x 100 ml), dried over Na_2SO_4 and the solvent removed to give a transparent oil. This oil was shaken in a Parr flask with Pd/C (10%, 300 mg) in MeOH (50 ml) overnight. The solid was removed by filtration and the solvent removed *in vacuo* to give a white solid corresponding to the title compound (7-2) (4.5 g, quantitative yield).

Example 38Preparation of N-[(4-Trifluoracetamidomethyl)phenyl]-N'-N''-bis(tert-butoxycarbonyl) guanidine (7-3)

5 4-Trifluoracetamidomethylaniline (7-2) (279 mg, 1.28 mmol) was added to a stirring mixture of N-N'-Di-Boc-N''-trifluoromethanesulfonyl-guanidine (prepared according to the procedure described in *J. Org. chem.* 1998, 63, 3804-3805) (500 mg, 1.28 mmol), TEA (108 µl, 1.28 mmol) in CH₂Cl₂ (10 ml). The reaction mixture was stirred for 6 hours. The mixture was diluted with CH₂Cl₂ (30 ml) and washed with HCl (1M, 20 ml), brine (20 ml), dried over Na₂SO₄ and the solvent removed *in vacuo* to give a solid. Column chromatography (CH₂Cl₂/MeOH, 99:1) gave a white solid corresponding to the title compound (350 mg, 52%).

10

15

Example 39Preparation of N-[(4-aminomethyl)phenyl]-N'-tert-butoxycarbonylguanidine (7-4)

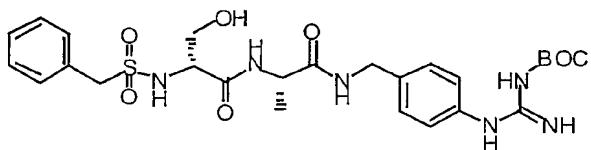
20 Potassium carbonate (500 mg) was added to a stirring solution of N-[(4-trifluoracetamidomethyl)phenyl]-N'-N''-bis(tert-butoxycarbonyl) guanidine (7-3) (300 mg, 0.833 mmol) in H₂O/MeOH (2:15, 17 ml) and the mixture was stirred overnight. The solvent was removed *in vacuo*, and the remaining residue was dissolved in H₂O (10 ml) and extracted

25

with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9:1, 3 x 10 ml). The organic layers were dried over Na_2SO_4 and removed *in vacuo* to give a white solid corresponding to the title compound (150 mg, 68%).

Example 40

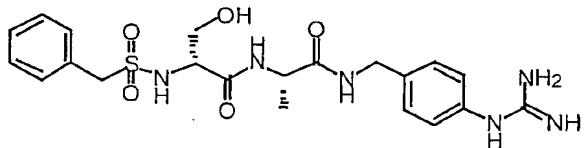
- 5 Preparation of Benzylsulfonyl-D-serine-L-alanine-(4-(N-tert-butoxycarbonylguanidino)benzylamide (7-5)



- A solution of N-[(4-aminomethyl)phenyl]-N'-tert-butoxycarbonylguanidine (7-4) (36 mg, 0.14 mmol),
 10 benzylsulfonyl-D-serine-L-alanine carboxylate (50 mg, 0.13 mmol), HATU (74 mg, 0.20 mmol), HOAT (27 mg, 0.20 mmol), and DIEA (68 μl , 0.39 mmol) in acetonitrile (2.0 ml) was stirred at room temperature overnight. The solution was diluted with EtOAc (20 ml) washed with HCl (1M, 10 ml), NaHCO_3 (saturated, 10 ml), brine (10 ml), and removed *in vacuo* to give an oil. HPLC purification ($\text{CH}_3\text{CN}, \text{H}_2\text{O}, 0.1\% \text{TFA}$) gave a fluffy white solid as the title compound (35 mg, 45%), MS (electrospray) 577 ($M + 1$).

Example 41

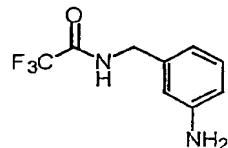
- 20 Preparation of Benzylsulfonyl-D-serine-L-alanine-4-guanidinobenzylamide (7-6)



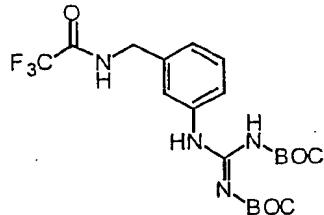
A solution benzylsulfonyl-D-serine-L-alanine-(4-(N-tert-butoxycarbonylguanidino)benzylamide (7-5) (9.0 mg, 0.016 mmol), in a mixture of CH₂Cl₂/TFA (1:1, 600 µl) was stirred at room temperature for 90 minutes. The solvent was 5 removed *in vacuo* to give a transparent oil. HPLC purification (CH₃CN, H₂O, 0.1% TFA) gave a fluffy white solid as the title compound (5.0 mg, 66%), MS (electrospray) 477 (M + 1).

Example 42

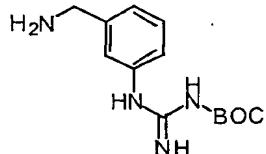
10 Preparation of 3-Trifluoracetamidometylaniline (8-2)



3-Nitrobenzylamine (8-1) (3.0 g, 16 mmol) was added in portions to stirring trifluoroacetic anhydride (30 ml) while the mixture was being cooled on ice, and the mixture stirred 15 overnight. The suspension was poured onto ice (approximately 200 g) and the cloudy suspension was extracted with CH₂Cl₂ (2 x 100 ml) dried over Na₂SO₄ and the solvent removed to give a transparent oil. This oil was shaken in a Parr flask with Pd/C (10%, 300 mg) in MeOH (30 20 ml) overnight. The solid was removed by filtration and the solvent removed *in vacuo* to give a white solid corresponding to the title compound (3.3 g, 95%).

Example 43Preparation of N-[(3-Trifluoracetamidomethyl)phenyl]-N'-N''-bis(tert-butoxycarbonyl) guanidine (8-3)

5 3-Trifluoracetamidomethylaniline (8-2) (the product of Example 42) (500 mg, 2.29 mmol) was added to a stirring mixture of N-N'-Di-Boc-N''-trifluoromethanesulfonyl-guanidine (prepared according to the procedure described in *J. Org. chem.* 1998, 63, 3804-3805) (986 mg, 2.52 mmol), TEA (642 µl, 4.58 mmol) in CH₂Cl₂ (10 ml), and the mixture was stirred for 24 hours. The mixture was washed with HCl (1M, 10 ml), brine (10 ml) dried over Na₂SO₄ and the solvent removed *in vacuo* to give a solid. Column chromatography (CH₂Cl₂/MeOH, 98:2) gave a white solid corresponding to the title compound (479 mg, 55%). MS (electrospray) 461 (M + 1).

Example 44Preparation of N-[(3-aminomethyl)phenyl]-N'-tert-butoxycarbonylguanidine (8-4)

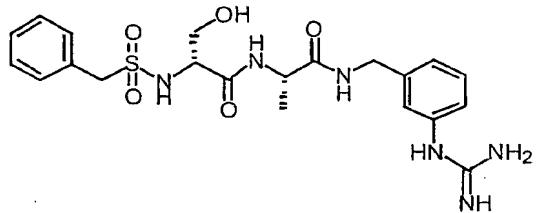
20

Potassium carbonate (1.0 g) was added to a stirring solution of N-[(3-trifluoracetamidomethyl)phenyl]-N'-N''-

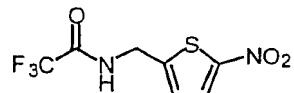
bis(tert-butoxycarbonyl) guanidine (**8-3**) (the product of Example 43) (450 mg, 0.978 mmol) in H₂O/MeOH (1:1, 4 ml) and the mixture was stirred overnight. The solvent was removed *in vacuo*, and the remaining residue was dissolved in H₂O (10 ml) and extracted with CH₂Cl₂/MeOH (9:1, 3 x 10 ml). The organic layers were dried over Na₂SO₄ and removed *in vacuo* to give a white solid corresponding to the title compound (232 mg, 90%).

Example 45

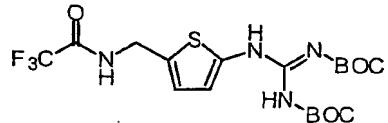
- 10 Preparation of Benzylsulfonyl-D-serine-L-alanine-3-guanidinobenzylamide (**8-5**)



A solution of N-[(3-aminomethyl)phenyl]-N'-tert-butoxycarbonylguanidine (**8-4**) (the product of Example 44) (130 mg, 0.492 mmol), benzylsulfonyl-D-serine-L-alanine carboxylate (190 mg, 0.492 mmol), HATU (380 mg, 0.739 mmol), HOAT (136 mg, 0.739 mmol), and DIEA (258 µL, 1.48 mmol) in acetonitrile (2.0 ml) was stirred at room temperature overnight. The solution was diluted with EtOAc (20 ml), washed with HCl (1M, 10 ml), NaHCO₃ (saturated, 10 ml), brine (10 ml), and removed *in vacuo* to give an oil. The oil was treated with a mixture of CH₂Cl₂/TFA (1:1, 3 ml) was stirred at room temperature for 2 hours. The solvent was removed *in vacuo* to give a transparent oil. HPLC purification (CH₃CN, H₂O, 0.1% TFA) gave a fluffy white solid as the title compound (80 mg, 34%), MS (electrospray) 477 (M + 1).

Example 46Preparation of 2-Nitro-5-(4-trifluoracetamidomethyl)-thiophene (9-2)

5 2-Aminomethylthiophene (9-1) (5.0 g, 44 mmol) was added in portions to stirring trifluoroacetic anhydride (20 ml) while the mixture was being cooled on ice, and the mixture stirred for one hour. To this solution was added KNO₃ (8.9 g, 88 mmol) in portions at -20°C. The homogeneous solution
 10 was warmed up to ambient temperature and stirred overnight. The resulting suspension was poured onto ice (approximately 200 g), and extracted with CH₂Cl₂ (2 x 100 ml), dried over Na₂SO₄, and the solvent removed to give a solid. Column chromatography (CH₂Cl₂) gave a white solid corresponding to
 15 the title compound (3.3 g, 29%), MS (electrospray): 255 (M + 1).

Example 47Preparation of N-[2-(5-Trifluoracetamidomethyl)thiophenyl]-N'-N''-(bis-tert-butoxycarbonyl) guanidine (9-3)

20

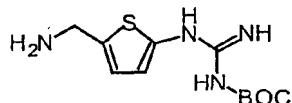
2-Nitro-5-(4-trifluoracetamidomethyl)-thiophene (9-2) (1.0 g, 3.9 mmol) was added to a saturated solution of HCl in MeOH at 0°C. SnCl₂ (4.4 g) was added in portions over 15 minutes and the mixture was stirred for 30 minutes. The
 25 solution was concentrated and then diluted with NaHCO₃ (10

ml). This solution was extracted with CH₂Cl₂ (2 x 20 ml), dried over Na₂SO₄ and the solvent removed *in vacuo* to give 2-amino-5-(4-trifluoracetamidomethyl)-thiophene as a yellow oil (>95% purity by NMR, 0.80 g, 95%). This compound was used
5 immediately in the next step without further purification.

2-Amino-5-(4-trifluoracetamidomethyl)-thiophene (0.80 g, 3.6 mmol) was added to a stirring solution of N-N'-Di-Boc-N''-trifluoromethanesulfonyl-guanidine (1.5 g, 3.8 mmol) and TEA (1.1 ml, 7.8 mmol) in CH₂Cl₂ (20 ml). The mixture was
10 stirred for 24 hours. The mixture was diluted with CH₂Cl₂ (20 ml) and washed with HCl (1M, 20 ml), brine (20 ml), dried over Na₂SO₄. The solvent was removed *in vacuo* to give an oil. Column chromatography (CH₂Cl₂/MeOH, 98:2) gave an oil corresponding to the title compound (9-3) (150 mg, 9%),
15 MS (electrospray): 467 (M + 1).

Example 48

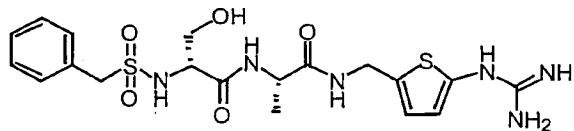
Preparation of N-[5-(4-aminomethyl)thiophenyl]-N'-(tert-butoxycarbonyl) guanidine (9-4)



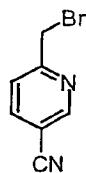
20 Potassium carbonate (500 mg) was added to a stirring solution of N-[2-(5-trifluoracetamidomethyl)thiophenyl]-N'-N''-(bis-tert-butoxycarbonyl) guanidine (9-3) (150 mg, 0.322 mmol) in H₂O/MeOH (1:1, 4 ml) and the mixture was stirred overnight. The solvent was removed *in vacuo*. The remaining residue was dissolved in H₂O (10 ml) and extracted with CH₂Cl₂/MeOH (95:5, 3 x 10 ml). The organic layers were dried over Na₂SO₄. Solvent was removed *in vacuo* to give a yellow solid corresponding to the title compound (150 mg, 68%).
25

Example 49Preparation of Benzylsulfonyl-D-Serine-L-Alanine-[5-(2-guanidino)thiophene] (9-5)

5



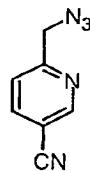
A solution of N-[5-(4-aminomethyl)thiophenyl]-N'-(tert-butoxycarbonyl) guanidine (9-4) (77 mg, 0.29 mmol), benzylsulfonyl-D-Serine-L-alanine carboxylate (110 mg, 0.285 mmol), HATU (162 mg, 0.427 mmol), HOAT (58 mg, 0.42 mmol), and DIEA (199 µL, 1.13 mmol) in acetonitrile (3.0 ml) was stirred at ambient temperature overnight. The solution was diluted with EtOAc (20 ml) washed with HCl (1M, 10 ml), NaHCO₃ (saturated, 10 ml), and brine (10 ml). The organic layer was dried over Na₂SO₄ and solvent was removed *in vacuo* to give an oil. To this oil was added TFA/CH₂Cl₂ (1:1, 2 ml); the mixture was stirred for 3 hours. HPLC purification (CH₃CN, H₂O, 0.1% THF) gave a fluffy white solid corresponding to the title compound (3 mg), MS (electrospray): 483 (M + 1).

20 Example 50Preparation of 6-Bromomethylnicotinonitrile (11-2)

To a solution of 6-Methylnicotinonitrile (**11-1**) (Lancaster) (15 g, 127 mmol) in carbon tetrachloride (300 ml) was added N-bromosuccinimide (27.12 g, 152.4 mmol). The resulting solution was degassed and purged with nitrogen and 5 AIBN (2,2'-azobisisobutyronitrile) (2.08 g, 12.6 mmol) was added. After 7 hours at 85°C, another batch of AIBN (1.04 g) was added and stirring was continued for another hour. After removal of the solvent the crude product was subjected to flash column chromatography in ethylacetate/ hexanes to 10 yield 10 g pure material (40%).

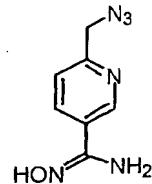
Example 51

Preparation of 6-azidomethylnicotinonitrile (**11-3**)

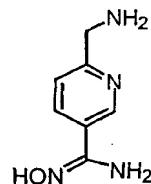


15

To a solution of 6-Bromomethylnicotinonirile (**11-2**) (8.0 g, 40.6 mmol) in DMF (100 ml) was added sodium azide (3.17 g, 48.8 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was 20 diluted with 400 ml diethylether and 100 ml water and the layers were separated. The aqueous layer was re-extracted with diethylether (2 x 100 ml). The combined ether extracts were washed with brine (2 x 100 ml) and then dried over MgSO₄ and filtered to yield 6.53 g product as a white solid.

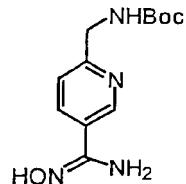
Example 52Preparation of 3-hydroxyamidino-6-azidomethylpyridine (11-4)

To a solution of the azide of Example 51 (11-3) (6.46 g, 40.6 mmol) in methanol (100 ml) was added hydroxylamine hydrochloride (3.95 g, 56.84 mmol), followed by triethylamine (9.6 ml). The solution was heated to 65°C for four hours. The solvent was removed under reduced pressure and the remaining residue was extracted with ethylacetate (300 ml) and water (100 ml). The water layer was re-extracted with ethylacetate (2 x 200 ml). The combined organic extracts were washed with brine (2 x 100 ml), dried over MgSO₄ and evaporated to yield the title product (11-4) (7.8 g).

15 Example 53Preparation of 3-hydroxyamidino-6-aminomethylpyridine (11-5)

20 To a solution of the azide (11-4) of Example 52 in THF/water (80 ml/ 5 ml) (7.8 g, 40.6 mmol) was added

triphenylphosphine (12.8 g, 48.72 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed to dryness and the remaining residue was diluted with 100 ml 1N HCl and 100 ml water. The aqueous 5 layer was extracted several times with dichloromethane. The aqueous layer was made basic (pH ~9) with an alkaline resin (Bio Rad AG 1-X8). The resin was filtered and washed thoroughly with water. The combined washings were dried under reduced pressure to give a white solid (6.60 g).

10 Example 54Preparation of 3-Hydroxyamidino-6-aminomethyl (Boc) pyridine (11-6)

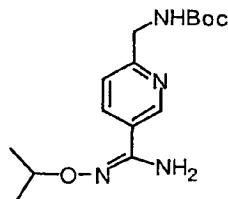
15

To a solution of the product of Example 53 (11-5) (0.5 g, 3.01 mmol) in dioxane and water (6 ml each) was added potassium carbonate (832 mg, 6.02 mmol), followed by Boc-anhydride (0.66 g, 3.01 mmol). The reaction mixture was 20 stirred overnight at room temperature. The reaction mixture was concentrated and the remaining residue was dissolved in ethylacetate and washed with sodium bicarbonate (saturated) and brine. The organic layer was dried over sodium sulfate, filtered and concentrated. Flash column chromatography in 25 8/2 ethylacetate/hexanes followed by ethylacetate and 9/1

dichloromethane/methanol afforded 0.43 g product as a white solid. NMR δ (ppm) CDCl₃: 8.8 (d, 1H), 7.9 (dd, 1H), 7.3 (d, 1H), 6.6 (bs, 1H), 5.5 (bs, 1H), 4.8 (bs, 1H), 4.4 (d, 2H), 1.4 (s, 9H).

5 Example 55

Preparation of 3-isopropoxyamidino-6-aminomethyl (Boc)pyridine (11-7)

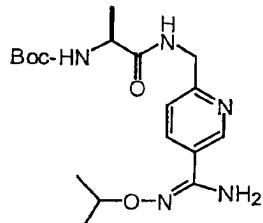


10

To a solution of the product of Example 54 (**11-6**) (0.43 g, 1.39 mmol) in dimethylformamide (5 ml) was added 2-iodopropane (210 μ l, 2.1 mmol), followed by cesium carbonate (0.68 g, 2.1 mmol). The reaction mixture was stirred
15 overnight at room temperature. The reaction mixture was diluted with ethylacetate and washed with sodium bicarbonate (saturated). The organic layer was dried over sodium sulfate, filtered and concentrated to give an orangish oil. Flash column chromatography performed in 1/1 ethylacetate/
20 hexane followed by ethylacetate yielded 229 mg (53%) product as a crystalline solid. NMR δ (ppm) CDCl₃: 8.8 (d, 1H), 7.9 (dd, 1H), 7.3 (d, 1H), 5.5 (bs, 1H), 4.7 (bs, 2H), 4.4 (d, 1H), 1.4 (s, 9H), 1.3 (d, 6H).

Example 56Preparation of Boc-Ala-3-isopropoxyloxyamidino-6-
aminomethylpyridine (11-8)

5

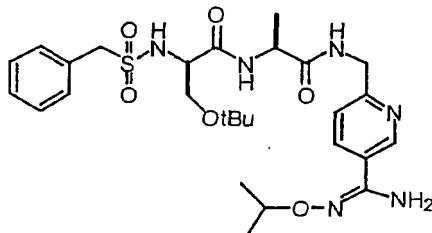


The compound of Example 55 (11-7) (229 mg, 0.74 mmol) was dissolved in dioxane (1 ml) and treated with a solution of 4N HCl in dioxane (1 ml) for one hour at room temperature. Removal of the solvent under reduced pressure afforded 290 mg of a white solid. That product was then dissolved in acetonitrile (4.12 ml), neutralized with diisopropylethylamine (538 µl, 3.09 mmol) and coupled to Boc-alanine (195 mg, 1.03 mmol) using EDC (197 mg, 1.03 mmol) and HOBT (157.6 mg, 1.03 mmol) as coupling reagents. After stirring overnight at room temperature, the solvent was removed under reduced pressure, and the remaining residue was diluted in ethylacetate. The organic layer was washed several times with sodium bicarbonate (saturated) and brine, dried over sodium sulfate, filtered and concentrated to a yellowish oil. Flash column chromatography in 9/1 ethylacetate/hexane, followed by ethylacetate afforded 181 mg of a white oil (46%). NMR δ(ppm) CDCl₃: 8.7 (d, 1H), 7.9 (dd, 1H), 7.3 (d, 1H), 7.1 (bs, 1H), 5.0 (bs, 1H), 4.7 (bs,

2H), 4.6 (d, 2H), 4.3 (m, 1H), 4.1 (m, 1H), 1.4 (s, 9H), 1.39 (d, 3H), 1.3 (d, 6H).

Example 57

Preparation of Benzylsulfonyl-dSer(tBu)-Ala-3-isopropoxyamidino-6-aminomethylpyridine (11-9)

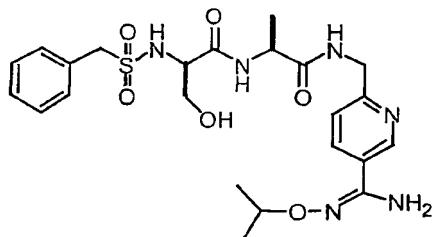


The compound of Example 56 (11-8) (181 mg, 0.48 mmol)
10 was dissolved in dioxane (1 ml) and treated with 4N HCl in
dioxane (1 ml) for 1.5 hour at room temperature. Removal of
the solvent under reduced pressure afforded 200 mg of a
white solid. That product was then dissolved in
acetonitrile (5 ml), neutralized with diisopropylethylamine
15 (298 µl, 1.71 mmol) and coupled to BnSO₂-dSer(tBu)-OH (180
mg, 0.57 mmol), using EDC (109 mg, 0.57 mmol) and HOBT (96
mg, 0.63 mmol) as coupling reagents. After stirring
overnight at room temperature, the solvent was removed under
reduced pressure, and the remaining residue was diluted in
20 ethylacetate. The organic layer was washed several times
with sodium bicarbonate (saturated) and brine, dried over
sodium sulfate, filtered and concentrated to give a solid
(250 mg, 76%). The product eluted at 11.5 minutes by reverse
phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75%

aqueous acetonitrile over 20 minutes. MS (electrospray):
577 (M + 1).

Example 58

Preparation of Benzylsufonyl-dSer-Ala-3-isopropoxyamidino-5
6-aminomethylpyridine (11-10)

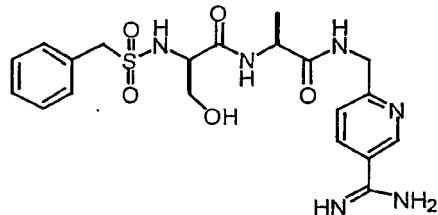


The compound of Example 57 (11-9) (137 mg, 0.24 mmol) was treated with 2 ml each dichloromethane and trifluoroacetic acid for one hour at room temperature.

- 10 Removal of the solvent under reduced pressure afforded 169 mg of an orangish oil. The product eluted at 9 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-90% aqueous acetonitrile over 20 minutes.

Example 59

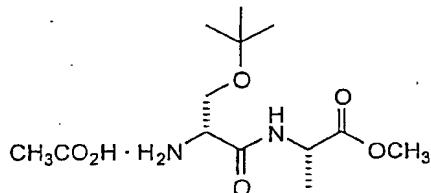
15 Preparation of Benzylsulfonyl-dSer-Ala-3-amidino-6-aminomethylpyridine (11-11)



The product of Example 58 (11-10) (74 mg, 0.14 mmol) in water (8 ml) and acetic acid (0.8 ml) was treated with activated zinc dust (91 mg) for 45 minutes. The solution 5 was filtered and the filtrate was subjected to purification by reverse phase HPLC (C18). The product eluted at 11 minutes by reverse phase HPLC at 0.1% trifluoroacetic acid in 5-25% aqueous acetonitrile over 20 minutes. MS (electrospray): 463 (M + 1).

10 B. Synthetic Routes For Certain Intermediate Compounds

(i) Examples 60 to 97 describe the synthesis of certain intermediates used in the preparation of compounds of the present invention. See also Figures 1 to 5.

Example 6015 Preparation of Synthesis of D-Ser(O-t-Bu)-Ala-OMe, acetate salt (1-3)

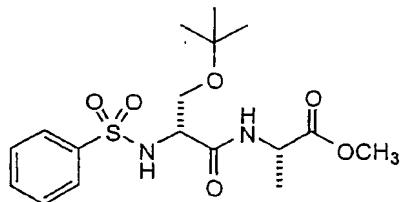
N- α -Cbz-D-serine (1-1) (Bachem, 4.97 g, 16.8 mmol), alanine methyl ester, hydrochloride salt (1-2) (Novabiochem, 20 4.7 g, 33.7 mmol), EDC (6.5 g, 33.7 mmol), and 1-hydroxybenzotriazole (2.6 g, 16.8 mmol) were combined, and acetonitrile (67 ml) was added. After stirring as a slurry for 10 minutes, diisopropylethylamine (14.4 ml, 84 mmol) was added, and the resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced 25 pressure.

pressure, and the residue was suspended in ethyl acetate (500 ml). The solution was washed with 0.5M HCl (2 x 100 ml), followed by saturated sodium bicarbonate (2 x 100 ml), and brine (100 ml). The organic layer was then dried with 5 sodium sulfate, and the solvent was removed *in vacuo* to afford Cbz-D-Ser(O-t-Bu)-Ala-OMe in a quantitative yield as a single peak by reverse-phase (C18) HPLC $t_R=16.9$ at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

10 Cbz-D-Ser(O-t-Bu)-Ala-OMe was then dissolved in ethanol/acetic acid/water (150 ml of a 4:1:1 mixture). The flask was charged with nitrogen, and 10% palladium on carbon (1.5 g) was added. This mixture was hydrogenated at 45 psi for 2 hours. The palladium catalyst was filtered, and 15 solvent removed under reduced pressure to give 4.58 g of the title compound in a 95% yield as a single peak by reverse-phase (C18) HPLC ($t_R=8.0$ minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes), and MS ($M+H=247.2$).

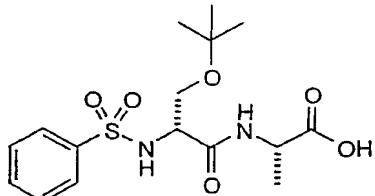
20 Example 61

Preparation of benzenesufonyl-D-Ser(O-t-Bu)-Ala-OMe (1-4)



To a stirred slurry of the compound of Example 60 (1-3) (1.0 g, 3.3 mmol) in acetonitrile (13 ml) was added 25 benzenesufonyl chloride (0.87 g, 4.9 mmol). To this mixture diisopropylethylamine (1.67 ml, 9.8 mmol) was added in five portions over a 1 hour period. The mixture was allowed to

stir an additional hour. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 ml). The solution was washed with 0.5M HCl (2 x 10 ml), followed by saturated sodium bicarbonate (2 x 10 ml), and brine (1 x 10 ml). The organic layer was then dried with sodium sulfate, and solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with 50% hexanes/ethyl acetate, yielding 0.54 g, 1.4 mmol, of product in a 43% yield. The product was a single peak by reverse phase (C18) HPLC ($t_R=20.2$ minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes). ^1H NMR (CD₃OD): 7.5-7.9 ppm (m, 5H), 4.3 ppm (q, 1H), 3.9 ppm (t, 1H), 3.7 (s, 3H), 3.4 ppm (m, 1H), 3.5 ppm (m, 1H), 1.3 ppm (d, 3H), 1.05 ppm (s, 9H).

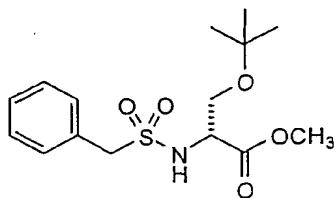
15 Example 62Preparation of benzenesufonyl-D-Ser(O-t-Bu)-Ala-OH (1-5)

To the compound of Example 61 (1-4) (0.53 g, 1.4 mmol) in methanol (9 ml) was added 1.0M lithium hydroxide (3.0 ml, 20 3 mmol). After stirring for 18 hours, the reaction mixture was poured over a column of 10 ml of DOWEX (50 X 8-400) ion exchange resin, and eluted with methanol/water (60 ml of a 1:1 mixture). The methanol was pumped off under reduced pressure and the remaining water was lyophilized, yielding 25 0.49 g, 1.3 mmol (95%) of the title compound as a single peak by reverse-phase (C18) HPLC ($t_R=13.5$ minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20

minutes). ^1H NMR (CD_3OD): 7.9 ppm (d, 2H), 7.6 ppm (t, 1H), 7.5 ppm (t, 2H), 4.25 ppm (q, 1H), 3.9 ppm (t, 1H), 3.5 ppm (m, 1H), 3.4 ppm (m, 1H), 1.3 ppm (d, 3H), 1.075 ppm (s, 9H).

Example 63

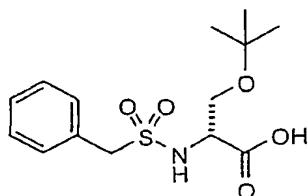
5 Preparation of benzylsulfonyl-D-Ser(O-t-Bu)-OMe



To a stirred solution of D-serine(O-t-Bu) methyl ester, hydrochloride salt (2.07 g, 9.8 mmol) in acetonitrile (39 ml) was added α -toluenesulfonyl chloride (1.86 g, 9.8 mmol).
 10 To this mixture of diisopropylethylamine (3.7 ml, 21.5 mmol) were added in five portions over a 1 hour period. The mixture was allowed to stir an additional hour. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 ml). The solution was washed with 0.5M HCl (2 x 10 ml), followed by saturated sodium bicarbonate (2 x 10 ml), and brine (1 x 10 ml). The organic layer was dried with sodium sulfate, and solvent was removed in vacuo to give 2.84 g of the title compound in 88% yield. $R_f=0.4$ (4:1 ethyl acetate:hexanes).

20 Example 64

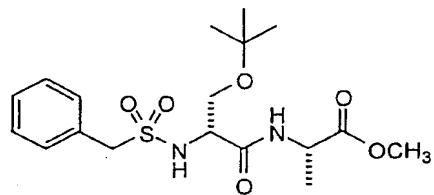
Preparation of benzylsulfonyl-D-Ser(O-t-Bu)-OH



To a stirred solution of the compound of Example 63 (2.66 g, 8.1 mmol) in methanol (54 ml) was added 1.0M lithium hydroxide (17.8 ml, 17.8 mmol). The reaction mixture was allowed to stir for 18 hours, then poured over a column of 10 ml of DOWEX(50 X 8-400) ion exchange resin and eluted with methanol:water (60 ml of a 1:1 mixture). The methanol was removed under reduced pressure, and the remaining aqueous solution was lyophilized to afford 2.47 g of the title compound in 97% yield. $t_R=14.8$ minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 65

Preparation of benzylsulfonyl-D-Ser(O-t-Bu)-Ala-OMe

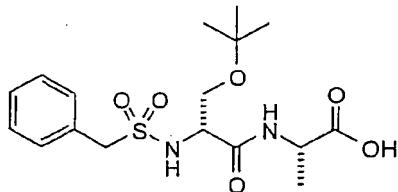


The compound of Example 64 (1.0 g, 3.2 mmol), alanine methyl ester, hydrochloride salt (Novabiochem, 0.89 g, 6.3 mmol), EDC (1.22 g, 6.3 mmol), and 1-hydroxybenzotriazole (0.49 g, 3.2 mmol) were combined and acetonitrile (13 ml) was added. After stirring the resulting slurry for 10 minutes, diisopropylethylamine (2.71 ml, 15.8 mmol) was added and the resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 ml). The solution was washed with 0.5M HCl (2 x 10 ml), followed by saturated sodium bicarbonate (2 x 10 ml), and brine (1 x 10 ml). The organic layer was then dried with sodium sulfate, and solvent was removed *in vacuo* to

afford 1.22 g of the title compound in 97% yield. $t_R=16.2$ minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 66

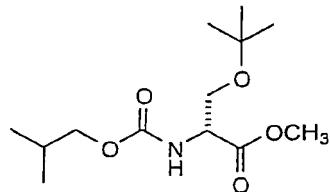
5 Preparation of benzylsulfonyl-D-Ser(O-t-Bu)-Ala-OH



To the compound of Example 65 (1.22 g, 3.1 mmol) in methanol (22 ml), was added 1M lithium hydroxide (7.2 ml, 7.2 mmol). After stirring 18 hours, the reaction mixture 10 was poured over a column of 10 ml of DOWEX(50 X 8-400) ion exchange resin and eluted with methanol:water (60 ml of a 1:1 mixture). The methanol was removed under reduced pressure, and the aqueous solution was lyophilized to afford 1.16 g of the title compound in 91% yield. $t_R=13.2$ minutes 15 (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 67

Preparation of i-butoxycarbonyl-D-Ser(O-t-Bu)-OMe

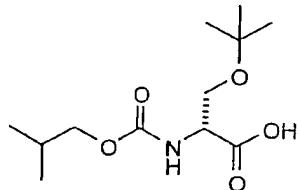


20 To a stirred homogeneous solution of HCl-D-Ser(O-t-Bu)-OMe (15 g, 71 mmol, Bachem) in tetrahydrofuran (200 ml), was added saturated sodium bicarbonate (80 ml), followed by isobutylchloroformate (19.45 g, 142 mmol). Layers were

separated and the aqueous layer was washed with ethyl acetate (50 ml). The organic phases were combined and solvent was removed under reduced pressure. The residue was suspended in ethyl acetate (100 ml) and washed with 1M HCl (100 ml), saturated sodium bicarbonate (100 ml), and brine (100 ml). The organic layer was dried with magnesium sulfate, treated with decolorizing charcoal, (such as that sold under the trade name Darco), filtered, and solvent removed under reduced pressure, giving quantitative yield of 10 the title compound. $R_f = 0.3$ (20% ethyl acetate/hexanes).

Example 68

Preparation of i-butoxycarbonyl-D-Ser(O-t-Bu)-OH



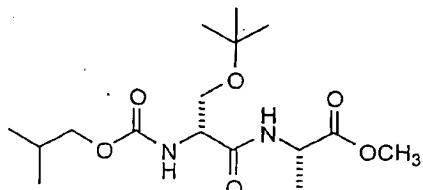
To a stirred solution of the compound of Example 67 (19.51 g, 70 mmol) in tetrahydrofuran (78 ml), was added lithium hydroxide (78 mmol, 3.28 g). The reaction mixture was stirred vigorously for 3 hours until no starting material was observed by TLC (20% ethyl acetate/hexanes). The solution was acidified with concentrated HCl to pH~2 and 20 the solvent removed under reduced pressure. The crude product was suspended in ethyl acetate and extracted with saturated sodium bicarbonate (2X, 75 ml). The combined sodium bicarbonate washes were acidified with 6M HCl and the oil that separated was extracted with ethyl acetate (2 x 100 ml). The combined organic layers were dried with magnesium sulfate, treated with Darco, filtered, and solvent removed 25

under reduced pressure, giving quantitative yield of the title compound. $R_f = 0.01$ (20% ethyl acetate in hexanes).

Example 69

Preparation of i-butoxycarbonyl-D-Ser(O-t-Bu)-Ala-OMe

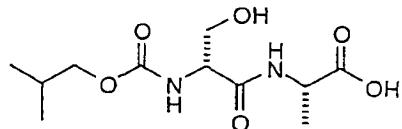
5



To a solution of the compound of Example 68 (16.5 g, 63 mmol), HCl-Ala-OMe (10.6 g, 76 mmol), 1-hydroxybenzotriazole (10.2 g, 76 mmol), and EDC (16.33 g, 85 mmol) in acetonitrile (280 ml) at 0°C, was added 4-methylmorpholine (35 ml, 315 mmol). This mixture was stirred for 1 hour at 0°C, then at ambient temperature for 72 hours. The solvent was removed under reduced pressure and the resulting residue was suspended in ethyl acetate (300 ml) and 1M HCl (350 ml). The aqueous layer was separated and washed with ethyl acetate (300 ml). The combined ethyl acetate layers were combined and washed with 1M HCl (300 ml), saturated sodium bicarbonate (400 ml), and brine (200 ml). The organic layer was dried with magnesium sulfate, treated with Darco decolorizing charcoal and filtered. The solvent was removed under reduced pressure, yielding 21.48 g of the title compound (98% yield).

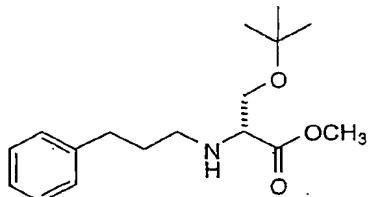
Example 70

Preparation of i-butoxycarbonyl-D-Ser-Ala-OH

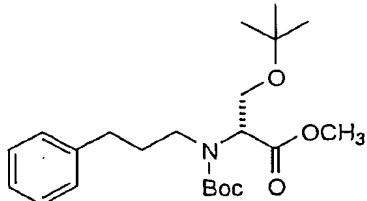


The compound of Example 69 (21 g, 58 mmol) was dissolved in trifluoroacetic acid (110 ml); the resulting mixture was stirred for 35 minutes. The solution was cooled in an ice bath, saturated sodium bicarbonate (630 ml) was 5 added, followed by solid sodium bicarbonate (70 g) over 45 minutes to a resulting pH=7. The aqueous solution was extracted with ethyl acetate (3 x 250 ml). The combined organic extracts were combined, dried with magnesium sulfate, treated with Darco decolorizing charcoal and 10 filtered. The solvent was removed in vacuo, giving a quantitative yield of i-butoxycarbonyl-D-Ser-Ala-OMe.

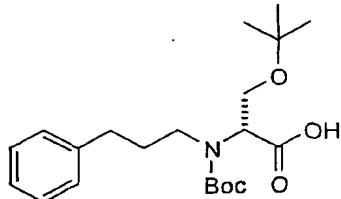
To a stirred solution of the crude residue in tetrahydrofuran (68 ml), was added lithium hydroxide (2.7 g, 64 mmol, 1.1 eq.) in water (17 ml). The reaction mixture 15 was stirred vigorously for 0.5 hours until there was no more of the starting material observed by TLC (9:1 dichloromethane/isopropanol). The solution was acidified with 6M HCl (13 ml) to pH~2 and the solvent was removed under reduced pressure. The crude product was suspended in 20 ethyl acetate (400 ml) and water (50 ml). The aqueous layer was extracted with ethyl acetate (200 ml). The combined organic layers were dried with magnesium sulfate, filtered, and the solvent removed in vacuo, yielding 13.94 g of the title compound (86% yield). $R_f = 0.3$ (90:30:5 chloroform/methanol/ acetic acid).

Example 71Preparation of N- α -(3-phenylpropyl)-D-serine-t-butyl ether methyl ester

5 Serine O-t-butyl ether methyl ester (1.50 g, 7.1 mmol), hydrocinnamaldehyde (1.40 ml, 10.6 mmol), and triethylamine (1.18 ml, 8.5 mmol) were refluxed in tetrahydrofuran (70 ml) for 4 hours. After the solution was allowed to cool to room temperature, sodium borohydride (0.46 g, 12 mmol) was added
10 in two portions to the stirred solution. The reaction mixture was stirred at ambient temperature for 30 minutes; the solution was concentrated under reduced pressure. The residue was partitioned between ethyl acetate 1.0M HCl. The organic layer was washed with 1.0N HCl. The aqueous layer
15 was basified with 40% NaOH to pH 10, then extracted with ethyl acetate (2X). The combined organic layers were dried over sodium sulfate; the solvent was removed in vacuo. The residue was purified by flash chromatography over silica gel (1 x 6 inch column) eluting with 10-30% ethyl
20 acetate/hexanes to afford 150 mg (7% yield) of the title compound. $R_f = 0.60$ (50% ethyl acetate/hexanes).

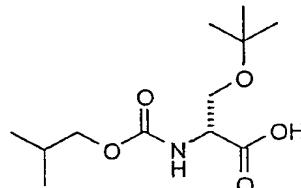
Example 72Preparation of N- α -t-butoxycarbonyl-N- α -(3-phenylpropyl)-D-serine-t-butyl ether methyl ester

5 The compound of Example 71 (150 mg, 0.51 mmol), di-t-butylidicarbonate (167 mg, 0.77 mmol) and
diisopropylethylamine (0.13 ml, 0.77 mmol) were stirred in
tetrahydrofuran (2 ml) at ambient temperature overnight.
The reaction mixture was concentrated under reduced
10 pressure. The residue was diluted with ethyl acetate (20
ml), washed successively with 1.0N HCl (2X), saturated
sodium bicarbonate (2X), and brine (1X). The solvent was
removed in vacuo to afford 206 mg of the title compound in
quantitative yield. $R_f = 0.74$ (50% ethyl acetate/hexanes).

Example 73Preparation of N- α -t-butoxycarbonyl-N- α -(3-phenylpropyl)-D-serine-t-butyl ether

15 To a solution of the compound of Example 72 (206 mg,
20 0.52 mmol) in methanol (3.5 ml), was added dropwise 1.0N
LiOH (0.63 ml, 0.63 mmol). The solution became cloudy, then
became homogeneous in 5 minutes. The reaction mixture was
allowed to stir at ambient temperature overnight.

Additional 1.0N LiOH (1.47 ml) was added. After 2 hours, additional 1.0N LiOH (1.0 ml) was added. After no starting material was observed by TLC (50% ethyl acetate/hexanes), the reaction mixture was acidified to pH4 with DOWEX (50 X 8-400) ion exchange resin. The solution was filtered, rinsing with methanol, then water. The solution was concentrated under reduced pressure, then lyophilized to afford 189 mg of the title compound in 95% yield as a yellow oil. $R_f = 0.04$ (50% ethyl acetate/hexanes).

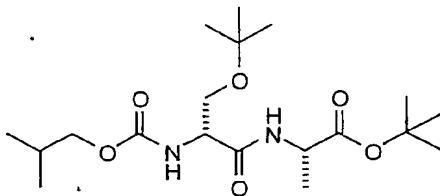
10 Example 74Preparation of i-butoxycarbonyl-D-Ser(O-t-butyl)-OH (2-2)

To a solution of D-serine-O-t-butyl ether (2-1) (10.5 g, 65.3 mmol) in water (51 ml) was added sodium carbonate (20.1 g, 196.2 mmol) followed by isobutyl chloroformate (9.8 ml, 75.2 mmol). After 3 hours this mixture became cloudy and was stirred for an additional 3 hours. After 6 hours the solution was acidified with 6M HCl (~50 ml). The reaction mixture was then extracted with ethyl acetate (3 x 200 ml). After the initial extraction, the aqueous layer was saturated with sodium chloride and extracted with ethyl acetate (2 x 200 ml). The ethyl acetate layers were combined and dried with sodium sulfate, followed by removal of the solvent *in vacuo*, yielding 17.0 g (99.5% yield) of the title compound as a white solid. HPLC: t=18.5 minutes in a 5% to 75% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom

pore, C18 column at a 1 ml/minute flow rate. NMR(CDCl₃): 5.5ppm(m, 1H), 4.45ppm(bs, 1H), 3.82-3.95ppm(m, 3H), 3.52-3.6ppm(m, 1H), 1.85-1.97ppm(m, 1H), 1.2ppm(s, 9H), 0.9ppm(d, 6H).

5 Example 75

Preparation of i-butoxycarbonyl-D-Ser(t-Bu)-L-Ala-O-t-Bu (2-3)



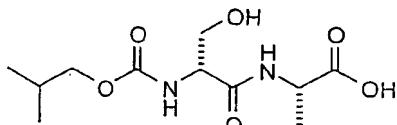
A solution of the compound of Example 74 (2-2) (10 g,
10 38.3 mmol), L-alanine t-butyl ester, HCl salt (10.43 g, 57.4
mmol), EDC (11.05 g, 57 mmol), and hydroxybenzotriazole
(5.85 g, 38.3 mmol) in acetonitrile (153 ml) was stirred for
15 minutes at room temperature. Diisopropylethylamine (32.7
ml, 191 mmol) was added and the reaction mixture stirred for
15 18 hours. The solvent was removed under reduced pressure;
the resulting residue was resuspended in ethyl acetate (1000
ml) and 1M HCl (100 ml). The ethyl acetate layer was washed
with 0.5M HCl (100 ml), saturated with sodium bicarbonate (2
x 100 ml), and brine (100 ml). The ethyl acetate layer was
20 dried with sodium sulfate and solvent was removed in vacuo,
resulting in a quantitative yield of title compound. HPLC:
tr=18.7 minutes in a 5% to 90% acetonitrile gradient in 0.1%
aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100
angstrom pore, C18 column at a 1 ml/minute flow rate.
25 NMR(CDCl₃): 7.15ppm(bs, 1H), 5.6ppm(bs, 1H), 4.4-4.5ppm(m,
1H), 4.2ppm(bs, 1H), 3.87-3.95ppm(m, 3H), 3.3-3.4ppm(m, 1H),

1.85-1.95 ppm (m, 1H), 1.45 ppm (s, 9H), 1.39 ppm (d, 3H),
1.2 ppm (s, 9H), 0.9 ppm (d, 6H).

Example 76

Preparation of i-butoxycarbonyl-D-Ser-L-Ala-OH (2-4)

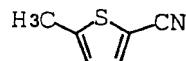
5



To a solution of the compound of Example 75 (2-3) (7.15 g, 18.4 mmol) in dichloromethane (35 ml), was added trifluoroacetic acid (35 ml). This mixture was stirred for two hours, then solvent was removed under reduced pressure. 10 Toluene was added and the solvents were removed in vacuo to remove the trifluoroacetic acid. A quantitative yield of the title compound as a viscous yellow oil was then carried on to the next step. $t_r=10.5$ minutes in a 5% to 90% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 15 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 ml/minute flow rate.

Example 77

Preparation of 2-cyano-5-methylthiophene (3-2)

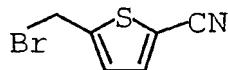


20 A solution of 2-bromo-5-methylthiophene (3-1) (TCI chemicals, 5 g, 28 mmol) and copper(I) cyanide (Aldrich, 2.53 g, 28 mmol) in DMF (10 ml) was heated at reflux for 4 hours. After cooling to ambient temperature, ethyl acetate (500 ml) and a 10% NaCN aqueous solution (500 ml) was added. 25 After separation of aqueous and organic layers, the aqueous layer was extracted with ethyl acetate (300 ml). The

combined organic layers were concentrated to an oil, which was further purified by a flash column chromatography (ethyl acetate) to give the title compound (3.03 g, 87%). TLC: R_f 0.30 (1:1 of hexane/ethyl acetate); ¹H NMR (CDCl₃): δ 2.55 (m, 3H), 6.76 (d, 1H, J = 3.6 Hz), 7.42 (d, 1H, J = 3.6 Hz).

Example 78

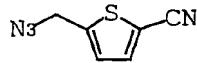
Preparation of 2-cyano-5-(bromomethyl)thiophene (3-3)



A solution of 2-cyano-5-methylthiophene (compound 3-2, 10 3.0 g, 24 mmol), N-bromosuccinimide (Aldrich, 4.8 g, 27 mmol), and 2,2'-azobisisobutyronitrile (Aldrich, 0.4 g, 2.4 mmol) in CCl₄ (Aldrich, 60 ml) was heated at reflux for 5 hours. After cooling to ambient temperature, the solvent was removed under vacuum to give a yellow oil. The oil was purified by a flash column chromatography (1:1 hexane/ethyl acetate) to give the title compound (4.5 g, 91%). TLC: R_f 0.91 (1:1 of hexane/ethyl acetate); ¹H NMR (CDCl₃): δ 4.66 (s, 2H), 7.10 (d, 1H, J = 3.8 Hz), 7.48 (d, 1H, J = 3.8 Hz).

Example 79

20 Preparation of 2-cyano-5-(azidomethyl)thiophene (3-4)

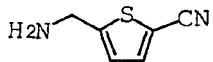


A solution of 2-cyano-5-(bromomethyl)thiophene (compound 3-3, 3.5 g, 17.3 mmol) and sodium azide (Aldrich, 1.7 g, 26 mmol) in DMF (Aldrich, 60 ml) was stirred at ambient temperature for 10 hours. Flash column chromatography (20% ethyl acetate in hexane) gave the title compound (2.35 g, 83%). TLC: R_f 0.48 (20% of ethyl acetate

in hexane); ^1H NMR (CDCl_3): δ 4.56 (s, 2H), 7.01 (d, 1H, J = 3.7 Hz), 7.55 (d, 1H, J = 3.7 Hz).

Example 80

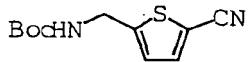
Preparation of 2-cyano-5-(aminomethyl)thiophene (3-5)



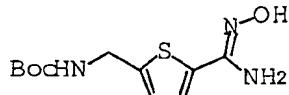
Triphenylphosphine (Aldrich, 5.7 g) was added to a solution of 2-cyano-5-(azidomethyl)thiophene (compound 3-4, 2.5 g, 10 mmol) in THF (Aldrich, 40 ml) and water (10 ml) at 0°C. The solution was allowed to warm to room temperature 10 and stirred at ambient temperature for 10 hours. RP-HPLC purification gave the title compound (2.3 g, 94%). MS (electrospray): 139 ($M + 1$); ^1H NMR (CDCl_3): δ 4.01 (s, 2H), 4.75 (br s, 2H, NH₂), 6.82 (d, 1H, J = 3.5 Hz), 7.08 (d, 1H, J = 3.5 Hz).

15 Example 81

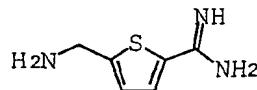
Preparation of 2-cyano-5-(t-butoxycarbonylaminomethyl)thiophene (3-6)



Potassium carbonate (Aldrich, 2 g) was added to a 20 solution of 2-cyano-5-(aminomethyl)thiophene (compound 3-5, 0.6 g, 4 mmol), Boc_2O (Fluka, 0.95 g, 4 mmol) in water (4 ml) and 1,4-dioxane (Aldrich). The resulting mixture was stirred at ambient temperature for 12 hours. Flash chromatography (1:1 hexane/ethyl acetate) gave the title 25 compound (0.58 g, 56%). MS (electrospray): 239 ($M + 1$); ^1H NMR (CDCl_3): δ 1.44 (s, 9H), 4.55 (s, 2H), 4.90 (br s, 1H, NH), 6.88 (d, 1H, J = 3.6 Hz), 7.07 (d, 1H, J = 3.6 Hz).

Example 82Preparation of 2-(N-hydroxyamidinyl)-5-(t-butoxycarbonylaminomethyl)thiophene (3-7)

5 A solution of 2-cyano-5-(t-butoxycarbonylmethyl)thiophene (compound 3-6, 560 mg, 2.44 mmol), hydroxylamine hydrochloride (Aldrich, 330 mg, 4.8 mmol) and 4-methylmorpholine (Aldrich, 1 ml, 9.1 mmol) in methanol (5 ml) was stirred at ambient temperature for 12 hours. Flash chromatography (5:95:1 isopropyl alcohol/methylene chloride/triethylamine) gave the title compound (550 mg, 86%). MS (electrospray): 272 (M + 1).

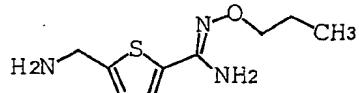
Example 83Preparation of 2-amidinyl-5-(aminomethyl)thiophene (3-8)

15 10% Pd-on-C (Aldrich, 100 mg) was added to a solution of 2-(N-Hydroxyamidinyl)-5-(t-butoxycarbonylaminomethyl)thiophene (compound 3-7, 900 mg, 3.3 mmol) in methanol (10 ml). The resulting mixture was 20 hydrogenated (45 psi of H₂) in a Parr apparatus at room temperature for 10 hours. The catalyst was removed by filtering and the solvent was evaporated under vacuum to give the Boc protected intermediate [900 mg, 94%, MS (electrospray) 256 (M + 1)] which was treated with 4 M HCl 25 in 1,4-dioxane (Aldrich, 5 ml) for 3 hours at ambient temperature to yield the title compound (460 mg, 84%). MS

(electrospray): 56 (M + 1); ^1H NMR (CD_3OD): δ 4.43 (s, 2H), 7.42 (d, 1H, J = 3.5 Hz), 7.78 (d, 1H, J = 3.5 Hz).

Example 84

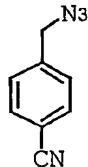
Preparation of 2-[N-(propyloxy)amidinyl]-5-(aminomethyl)thiophene (3-9)



CsCO_3 (Aldrich, 0.5 g) was added to a solution of 2-(N-Hydroxyamidinyl)-5-(t-butoxycarbonylaminomethyl)thiophene (compound 3-7, 271 mg, 1.0 mmol) and iodopropane (Aldrich, 10 200 mg, 1.2 mmol) in DMF. The reaction mixture was stirred at ambient temperature for 10 hours. Flash chromatography (5:95:1 isopropyl alcohol/methylene chloride/triethylamine) gave the Boc protected intermediate [MS (electrospray) 314 (M + 1)] which was treated with 4M HCl in 1,4-dioxane (Aldrich) for 3 hours at ambient temperature to yield the title compound (201 mg, 81%). MS (electrospray): 214 (M + 1); ^1H NMR (CDCl_3): δ 0.95 (t, 3H, J = 7.5 Hz), 1.55 (br s, 2H, NH₂), 1.70 (m, 2H), 4.00 (t, 2H, J = 7.5 Hz), 4.01 (d, 2H, J = 7.5 Hz), 4.70 (br s, 2H, NH₂), 6.80 (d, 1H, J = 3.6 Hz), 7.08 (d, 1H, J = 3.6 Hz).

Example 85

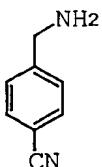
Preparation of α -azido-4-cyanotoluene (4-2)



Sodium azide (Aldrich, 3.5 g, 54 mmol) was added to a solution of p-cyanobenzyl bromide (Aldrich, 10 g, 51 mmol) in DMF (100 ml), and the resulting mixture was stirred at ambient temperature for 5 hours. The reaction mixture was 5 then diluted with water (350 ml) and extracted with ether (2 x 100 ml). Combined organic layers were washed with brine and dried (MgSO_4). Removal of solvent gave the title compound (8 g, 96%). ^1H NMR (CDCl_3): δ 4.42 (s, 2H), 7.41 (d, 2H, J = 8.1 Hz), 7.65 (d, 2H, J = 8.1 Hz).

10 Example 86

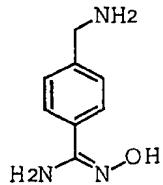
Preparation of p-cyanobenzylamine (4-3)



10% Pd-on-C (Aldrich, 800 mg) catalyst was added to a solution of α -azido-4-cyanotoluene (compound 4-2, 8 g, 51 mmol) in EtOAc (150 ml). The reaction mixture was 15 hydrogenated (H_2 , 45 psi) in a Parr apparatus for 11 hours. Catalyst was removed by filtering and the solvent was removed under vacuum to give the title compound (6.3 g, 93%). ^1H NMR (CDCl_3): δ 3.85 (s, 2H), 7.45 (d, 2H, J = 8.1), 20 7.60 (d, 2H, J = 8.1 Hz), 7.78 (s, 2H, NH₂).

Example 87

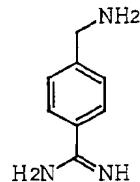
Preparation of 4-(aminomethyl)phenyl-N-hydroxyamidine (4-4)



Hydroxylamine hydrochloride (7 g) was added to a solution of compound 4-3 (7 g) and NMM (4 ml) in methanol (100 ml). The mixture was stirred at ambient temperature for 3 days. The compound was purified by RP HPLC to give 5 the title compound (7 g, 89%). MS (electrospray): 166 (M + 1).

Example 88

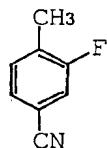
Preparation of 4-(aminomethyl)phenylamidine (4-5)



10 10% Pd-on-C (Aldrich, 800 mg) was added to a solution of 4-(aminomethyl)phenyl-N-hydroxyamidine (compound 4-4, 7 g) in methanol (150 ml). The reaction mixture was hydrogenated (H_2 , 45 psi) in a Parr apparatus for 48 hours. Catalyst was removed by filtering and the solvent was 15 removed under vacuum to give the title compound (6.3 g, 99%). MS (electrospray): 150 (M + 1).

Example 89

Preparation of 2-fluoro-4-cyanotoluene (5-2)

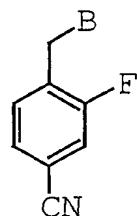


20 Copper(I) cyanide (Aldrich, 3.6 g, 40 mmol) was added to a solution of 4-bromo-2-fluorotoluene (Aldrich, 5 g, 27 mmol) in DMF (60 ml). The reaction mixture was heated at 150°C for 11 hours. After cooling to room temperature, the

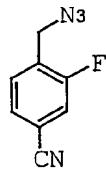
mixture was partitioned between water and EtOAc (500 ml each). The organic layer was dried ($MgSO_4$), and solvent was removed under vacuum to give the title compound (2.08, 58%).
 1H NMR ($CDCl_3$): δ 2.36 (s, 3H), 7.30 (m, 3H), 7.35 (d, 1H, J = 8.1 Hz).

Example 90

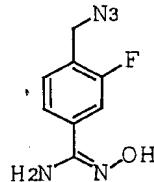
Preparation of 3-fluoro-4-(bromomethyl)benzonitrile (5-3)



NBS (Aldrich, 3.02 g, 17 mmol) and benzoylperoxide
10 (Aldrich, 0.37 g, 1.5 mmol) was added to a solution of 2-fluoro-4-cyanotoluene (compound 5-2, 2.08 g, 15 mmol) in CCl_4 . The reaction mixture was heated at 80°C for 14 hours. After cooling down to ambient temperature, the mixture was diluted with ether (100 ml) and washed with aqueous $Na_2S_2O_3$, and dried ($MgSO_4$). Removal of solvent under vacuum led to a yellow oil which was purified by flash chromatography. The title compound 5-3 (1.4 g, 42%) was obtained, together with a by-product, 3-fluoro-4-(bromomethyl)benzonitrile (5-4) (1.0 g, 30 %). For the title compound (5-3): 1H NMR ($CDCl_3$):
20 δ 4.46 (s, 2H), 7.35 (d, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 8.0 Hz), 7.52 (t, 1H, J = 8.0 Hz). For the by-product (5-4): 1H NMR ($CDCl_3$): δ 6.90 (s, 1H), 7.35 (d, 1H, J = 8.0 Hz), 7.55 (d, 1H, J = 8.0 Hz), 7.96 (t, 1H, J = 8.0 Hz).

Example 91Preparation of 3-fluoro-4-(azidomethyl)benzonitrile (5-5)

Sodium azide (Aldrich, 0.63 g, 9.8 mmol) was added to a
5 solution of 3-fluoro-4-(bromomethyl)benzonitrile (compound
5-3, 1.4 g, 6.5 mmol) in DMF (15 ml). After stirring at
ambient temperature for 20 hours, the reaction mixture was
partitioned in EtOAc and water (100 ml, each). Organic
layer was then dried (MgSO_4), and solvent was removed under
10 vacuum to give the title compound (0.995 g, 86%). ^1H NMR
(CDCl_3): δ 4.50 (s, 2H), 7.38 (d, 2H, J = 8.1 Hz), 7.52 (m,
2H).

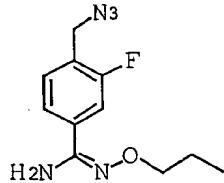
Example 92Preparation of 3-fluoro-4-(azidomethyl)phenyl-N-hydroxyamidine (5-6)

Hydroxylamine hydrochloride (Aldrich, 800 mg, 11.6
mmol) was added to a solution of 3-fluoro-4-(azidomethyl)benzonitrile (compound 5-5, 1.2 g, 6.8 mmol)
20 and NMM (2 ml) in methanol (25 ml). After stirring at room
temperature for 3 days, the reaction mixture was diluted
with EtOAc and washed with brine. Removal of solvent under

vacuum yielded the title compound (1.38, 82%). ^1H NMR (CD_3OD): δ 4.41 (s, 2H), 7.45 (m, 3H).

Example 93

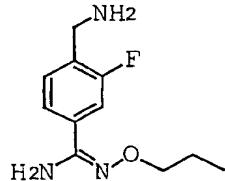
Preparation of 3-fluoro-4-(azidomethyl)phenyl(-N-
5 propyloxy)amidine (5-7)



Cesium carbonate (Aldrich, 3.2 g, 9.9 mmol) was added to a solution of iodopropane (1 ml, 10 mmol) and 3-fluoro-4-(azidomethyl)phenyl-N-hydroxyamidine (compound 5-6, 1.38 g, 6.6 mmol) in DMF (20 ml). The reaction mixture was heated at 50°C for 20 hours. After cooling down to ambient temperature, water was added and resulting mixture was extracted with ether. The organic layer was washed with brine and dried (MgSO_4). Flash chromatography gave the title compound (1.03 g, 62%). ^1H NMR (CDCl_3): δ 0.99 (t, 3H, $J = 7.5$ Hz), 1.75 (m, 2H), 4.08 (t, 2H, $J = 7.5$ Hz), 4.40 (s, 2H), 4.78 (br s, 2H), 7.40 (m, 3H).

Example 94

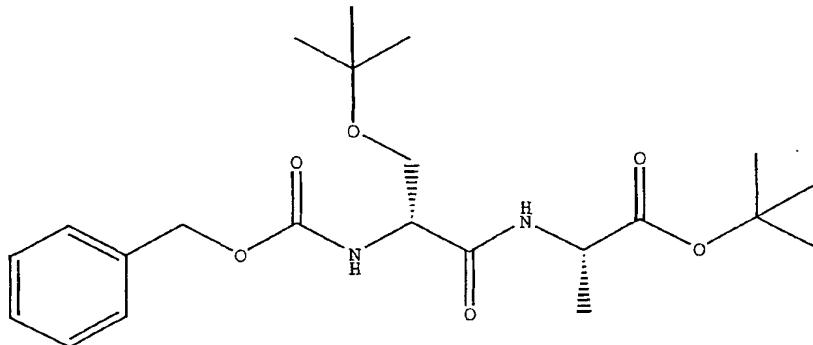
Preparation of 3-fluoro-4-(aminomethyl)phenyl(-N-
20 propyloxy)amidine (5-8)



Triphenylphosphine (Aldrich, 1.6 g, 6.2 mmol) was added to a solution of 3-fluoro-4-(azidomethyl)phenyl(-N-propyloxy)amidine (compound 5-7, 1.03 g, 4.1 mmol) in THF (15 ml). The reaction mixture was stirred at ambient 5 temperature for 20 hours. NaOH (3M) was added to the reaction mixture until pH = 14. The resulting solution was extracted with EtOAc (2 x 100ml). The combined organic layers were washed brine and dried ($MgSO_4$). Removal of solvent under vacuum gave the title compound (825 mg, 77%).
10 1H NMR (CD_3OD): δ 0.98 (t, 3H, J = 7.5 Hz), 1.72 (m, 2H), 3.82 (s, 2H), 3.95 (t, 2H, J = 7.5 Hz), 7.35 (d, 1H, J = 8.0 Hz), 7.40 (m, 2H).

Example 95

Preparation of N- α -benzyloxycarbonyl-D-Ser(O-t-butyl)-L-Ala
15 t-butyl ester



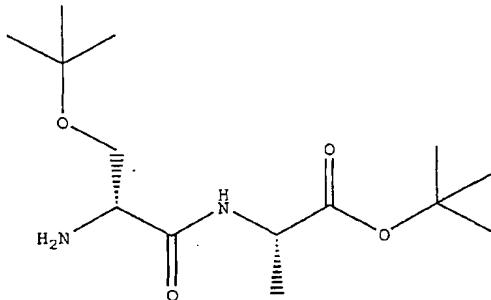
To a solution of N- α -Cbz-D-serine t-butyl ether (5.02 g, 17 mmol) in acetonitrile (100 ml) was added EDC (4.90 g, 25.5 mmol, 1.5 equiv) and 1-hydroxybenzotriazole (2.60 g, 17 mmol). After stirring for 45 minutes, alanine t-butyl 20 ester, HCl salt (3.55 g, 19.6 mmol, 1.15 equiv) and 4-methylmorpholine (7.5 ml, 68 mmol, 4 equiv) were added. The reaction mixture was stirred for 1.5 hours. TLC indicated the reaction was complete. The reaction mixture

was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 ml), then washed successively with 1N HCl, saturated sodium bicarbonate, water and brine (25 ml each). The solvent was removed in 5 vacuo to give an oil which crystallized on standing. The title compound (6.95 g) was obtained as a pale yellow solid in 97% yield. $R_f = 0.63$. (5% isopropanol in dichloromethane).

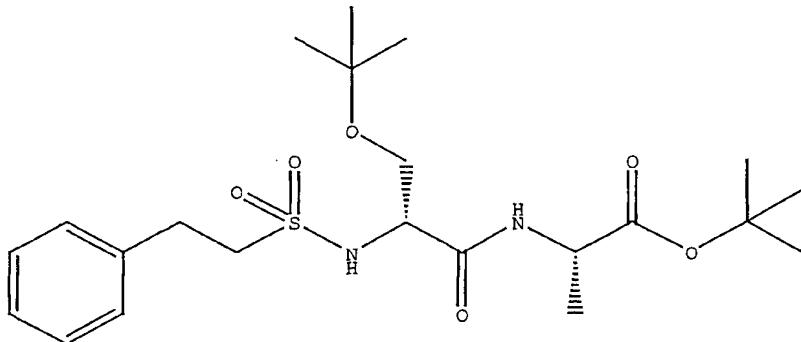
Example 96

Preparation of D-Ser(O-t-butyl)-L-Ala t-butyl ester

10



A solution of the compound of Example 95 (1.14 g, 2.69 mmol) in methanol was hydrogenated over palladium hydroxide (110 mg) at balloon pressure for 1.5 hours. The reaction mixture was filtered through celite, and the 15 solvent was removed in vacuo to afford the title compound in quantitative yield as a yellow oil. $R_f = 0.44$ (5% methanol in dichloromethane).

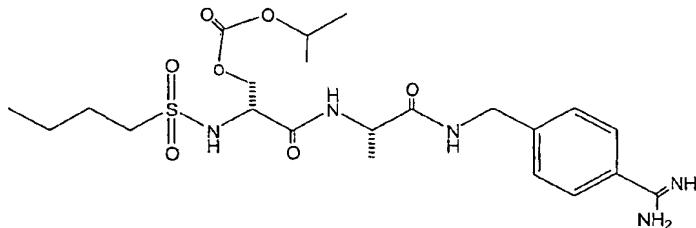
Example 97Preparation of phenethylsulfonyl-D-Ser(O-t-butyl)-L-Ala t-butyl ester

5 To a stirred solution of the compound of Example 96 (4.2 g, 14.6 mmol) and phenethylsulfonyl chloride (3.58 g, 17.5 mmol, 1.2 equiv) in acetonitrile (100 ml) cooled in an ice bath, was added 2,4,6-collidine (4.8 ml, 36.5 mmol, 2.5 equiv). The reaction mixture was allowed to warm to
10 room temperature, then stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (80 ml); the resulting solution was washed successively with 1.0N HCl, saturated sodium bicarbonate, water and brine, then dried
15 over sodium sulfate. The solvent was removed in vacuo. The residue was chromatographed through silica gel eluting with 0-60% ethyl acetate/hexanes. The title compound was isolated in 89% yield as a yellow oil. $R_f = 0.84$ (5% methanol in dichloromethane).

C. Synthetic Routes for Compounds Having Acyl or Carbonate Esters at P3

Example 98

5 Preparation of n-butylsulfonyl-D-(isopropoxycarbonyl)serine-alanine-4-amidinobenzylamide



To a solution of the compound of Example 9 (2.32 g, 4.3 mmol) in pyridine (100 ml) cooled in an ice bath, is added isopropyl chloroformate (21 ml of a 1M solution in 10 toluene, 21 mmol, 5 equiv). The reaction is monitored until determined to be complete by analytical HPLC. The reaction mixture is diluted with toluene (300 ml); the solvent is removed under reduced pressure. The residue is dissolved in acetonitrile (400 ml) and the solvent is removed under 15 reduced pressure. The residue is dissolved in acetonitrile (30 ml) and ethyl acetate (30 ml). Ether was added, and the precipitate was isolated. The solid is washed with ether, then dried in vacuo. The solid is washed with ether and 20 ethyl acetate (2 X 200 ml of a 1:1 mixture), then dried in vacuo to give the title compound.

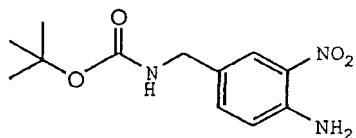
Example 99

Following the protocol of Example 98, with the noted substitution for isopropyl chloroformate, the following compounds are prepared:

Compound name	Substitution in Example 99
n-butylsulfonyl-D-[(+)-menthylloxycarbonyl]-serine-alanine-4-amidinobenzylamide	(+)-menthyl chloroformate
n-butylsulfonyl-D-(phenoxy carbonyl)-serine-alanine-4-amidinobenzylamide	phenyl chloroformate

Example 100Preparation of (4-Amino-3-nitro-benzyl)-carbamic acid tert-butyl ester

5



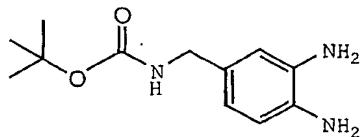
A portion of BH₃ in THF (1M, 40 mL) was added dropwise over 10 minutes to a stirring solution of 4-amino-3-nitro-5-benzonitrile (1.5 g, 9.2 mmol) in THF (20 mL) while being cooled in an ice bath. The yellow mixture which resulted
 10 was warmed up to room temperature, stirred for 2 hours and quenched with HCl (6M, 20 mL) at 0°C. The solvent was removed *in vacuo*; the residue was diluted with H₂O and the pH was adjusted to about 10 using NaOH (1M). The water layer was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic
 15 layers were dried over Na₂SO₄. The solvent was removed to give a yellow solid, 1.2 g (78%) which was used in the next synthesis step without further isolation.

The solid (500 mg, 3.0 mmol) was added to a homogeneous mixture of BOC-ON (754 mg, 3.0 mmol) in THF (10 mL). The resulting mixture was stirred at room temperature overnight. The mixture was diluted with aqueous NaHCO₃ and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried over NaSO₄. The solvent was removed *in vacuo* to give

an oil. Column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1) gave the title compound as yellow solid that solidified upon standing (760 mg, 95%), MS (electrospray) 268 ($M + 1$).

Example 101

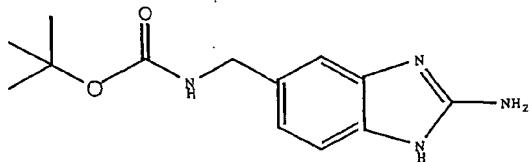
- 5 Preparation of (3,4-Diamino-benzyl)-carbamic acid tert-butyl ester



A mixture of (4-amino-3-nitro-benzyl)-carbamic acid 10
tert-butyl ester (500 mg, 1.87 mmol) and Pd (10% on carbon, 100 mg) in MeOH (10 mL) was shaken under H_2 (40 psi) for 3 hours. The catalyst was removed by filtration and the solvent was removed in vacuo to give an oil, which was used immediately for the next step (Example 102).

- 15 Example 102

Preparation of (2-Amino-1*H*-benzoimidazol-5-ylmethyl)-carbamic acid tert-butyl ester

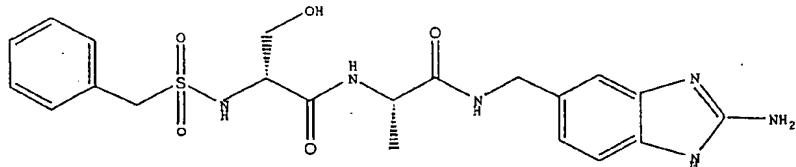


(3,4-Diamino-benzyl)-carbamic acid tert-butyl ester (500 mg, 20 2.10 mmol) was added to a stirring solution of CNBr (267 mg, 2.52 mmol) and NaHCO_3 (354 mg, 4.2 mmol) in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, 10 mL) and the mixture was stirred for 48 hours. The solvent was

removed in vacuo to give a white solid which was used in the next synthesis step without further isolation. MS (electrospray) 263 (M + 1).

Example 103

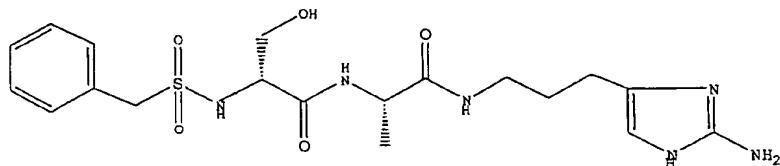
5 Preparation of Benzylsulfonyl-D-Serine-L-alanine-5-(2-aminobenzimidazolyl)methylamide



10 (2-Amino-1*H*-benzoimidazol-5-ylmethyl)-carbamic acid *tert*-butyl ester (50 mg, 0.3703 mmol) was added to a stirring solution of benzylsulfonyl-D-serine-L-alanine carboxylate (111 mg, 0.337 mmol), HATU (192 mg, 0.506 mmol), HOAT (69 mg, 0.506 mmol), and DIEA (174 μ L, 1.01 mmol) in acetonitrile (1.0 mL). The mixture was stirred room temperature overnight. HPLC purification (CH_3CN , H_2O , 0.1% TFA) gave a fluffy white solid as the title compound (25 mg, 24%), MS (electrospray) 475 (M + 1).

Example 104

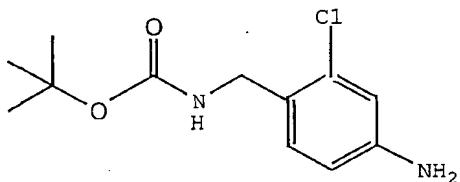
20 Preparation of Benzylsulfonyl-D-Serine-L-alanine-4-(2-aminoimidazolyl)-propylamide



4-(2-Aminoimidazolyl)-propylamine HCl (50 mg, 0.236 mmol) (prepared according to the procedure of Olofson et al. *J. Org. Chem.* 1998, 63, 1248) was added to a stirring solution of benzylsulfonyl-D-serine-L-alanine carboxylate (78 mg, 0.236 mmol), HATU (179 mg, 0.472 mmol), HOAT (64 mg, 0.472 mmol), and DIEA (164 µL, 0.943 mmol) in acetonitrile (1.0 mL). The mixture was stirred overnight at room temperature. HPLC purification (CH₃CN, H₂O, 0.1% TFA) gave a fluffy white solid as the title compound, MS (electrospray) 10 453 (M + 1).

Example 105

Preparation of (4-Amino-2-chloro-benzyl)-carbamic acid
tert-butyl ester



15 A portion of BH₃ in THF (1M, 36 mL) was added dropwise over 10 minutes to a stirring solution of 4-amino-2-chloro-5-benzonitrile (2.0 g, 13 mmol) in THF (10 mL) while being cooled in an ice bath. The yellow mixture which resulted was warmed up to room temperature, stirred for 2 hours and 20 quenched with HCl (6M, 20 mL) at 0°C. The solvent was removed *in vacuo*. The residue was diluted with H₂O and the pH was adjusted to about 10 using NaOH (1M). The water layer was extracted with CH₂Cl₂ (3 x 50 mL) and the combined organic layers were dried over Na₂SO₄. The solvent was 25 removed to give a yellow oil (2.5 g, 100%) which was used in the next synthesis step without further isolation.

The oil (500 mg, 2.6 mmol) (from the previous step) was added to a homogeneous mixture of BOC-ON (664 mg, 2.7 mmol) in THF (10 mL) and the resulting mixture was stirred at room temperature overnight. The mixture was diluted with aqueous 5 NaHCO₃ and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried over NaSO₄. The solvent was removed *in vacuo* to give a colorless oil. Column chromatography (CH₂Cl₂/MeOH, 99:1) gave the title compound as yellow solid that solidified upon standing, MS 10 (electrospray) 257 (M + 1).

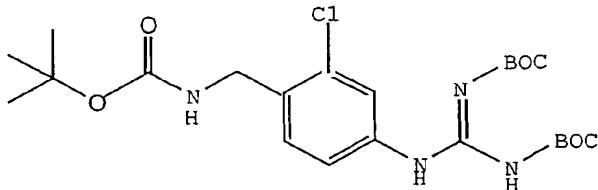
Example 106

Preparation of [(4-BOC-Aminomethyl)-3-chlorophenyl]-N'-

N''

-bis(tert-butoxycarbonyl) guanidine

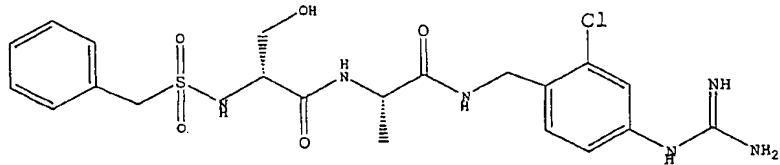
15



(4-Amino-2-chloro-benzyl)-carbamic acid *tert*-butyl ester (250 mg, 0.978 mmol), was added to a stirring mixture of N-N'-Di-Boc-N''-trifluoromethanesulfonyl-guanidine (343 mg, 0.879 mmol), TEA (108 μ L, 1.28 mmol) in CH₂Cl₂ (5 mL). 20 The mixture was stirred for 24 hours. The mixture was diluted with CH₂Cl₂ (20 mL) and washed with HCl (1M, 20 mL), brine (10 mL). The organic layer was dried over Na₂SO₄. The solvent was removed *in vacuo* to give a solid. Column chromatography (CH₂Cl₂/MeOH, 99:1) gave an oil corresponding 25 to the title compound (150 mg; 34%).

Example 107

Preparation of Benzylsulfonyl-D-Serine-L-alanine-4-guanidino-2-chlorobenzylamide

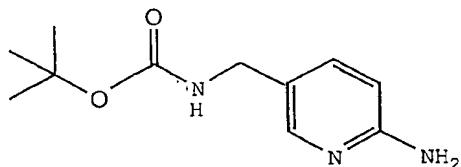


A solution of [(4-BOC-Aminomateoethyl)-3-chlorophenyl]-
 5 N'-N''-bis(tert-butoxycarbonyl) guanidine (100 mg, 0.201 mmol) in a mixture of CH₂Cl₂/TFA (1:1, 2 mL) was stirred at room temperature for 90 minutes. The solvent was removed *in vacuo* to give a transparent oil which was used in the next synthetic step without further solution.

10 The oil (from the previous step) was added to a stirring solution of benzylsulfonyl-D-serine-L-alanine carboxylate (69 mg, 0.209 mmol), HATU (157 mg, 0.418 mmol), HOAT (57 mg, 0.418 mmol), and DIEA (145 µL, 0.836 mmol) in acetonitrile (5.0 mL). The mixture was stirred room
 15 temperature overnight. HPLC purification (CH₃CN, H₂O, 0.1% TFA) gave the title compound as a fluffy white solid (25 mg, 24%), MS (electrospray) 511 (M + 1).

Example 108

Preparation of (6-Amino-pyridin-3-ylmethyl)-carbamic acid tert-butyl ester.

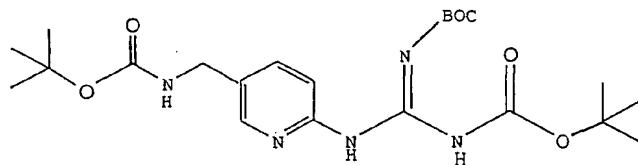


A portion of BH_3 in THF (1M, 40 mL) was added dropwise over 10 minutes to a stirring solution of 4-amino-2-chloro-5-benzonitrile (2.0 g, 14 mmol) in THF (5 mL) while being cooled in an ice bath. The yellow mixture which resulted
5 was warmed up to room temperature, stirred for 2 hours and quenched with HCl (6M, 20 mL) at 0°C. The solvent was removed *in vacuo*. The residue was diluted with H_2O and the pH was adjusted to about 10 using NaOH (1M). The water layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined
10 organic layers were dried over Na_2SO_4 ; the solvent was removed to give a solid (2.8 g, 100%).

This solid (2.0 g, 13 mmol) was added to a homogeneous mixture of BOC-ON (3.4 mg, 14 mmol) in CH_3CN (10 mL) and the mixture was stirred at room temperature overnight. The
15 mixture was diluted with aqueous NaHCO_3 and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over NaSO_4 . The solvent was removed *in vacuo* to give an colorless oil. Column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1)
gave the title compound as an oil that solidified upon
20 standing, MS (electrospray) 226 ($M + 1$).

Example 109

Preparation of [(3-BOC-Aminomethyl)-pyridinyl]-N'-N''-bis(tert-butoxycarbonyl) guanidine

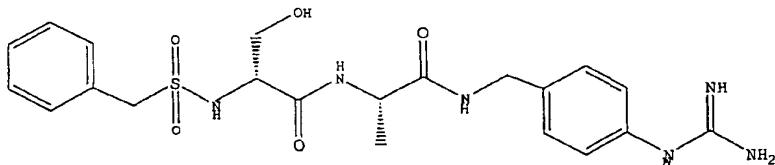


25 A portion of (6-Amino-pyridin-3-ylmethyl)-carbamic acid tert-butyl ester (400 mg, 1.78 mmol) was added to a stirring mixture of N-N'-Di-Boc-N''-trifluoromethanesulfonyl-

guanidine (765 mg, 1.96 mmol) and TEA (385 mL, 2.67 mmol) in CH₂Cl₂ (5 mL). The resulting mixture was stirred for 24 hours. The mixture was diluted with CH₂Cl₂ (20 mL) and washed with HCl (1M, 20 mL) and brine (10 mL). The organic layer was dried over Na₂SO₄ and the solvent was removed *in vacuo* to give a solid. Column chromatography (CH₂Cl₂/MeOH, 99:1) gave an oil corresponding to the title compound, MS (electrospray) 466 (M + 1).

Example 110

10 Benzylsulfonyl-D-Serine-L-alanine-4-guanidino-(2-pyridinyl)methylamide



A solution of [(3-BOC-Aminomateoethyl)-pyridinyl]-N'-N''-bis(tert-butoxycarbonyl) guanidine (100 mg, 0.215 mmol) in a mixture of CH₂Cl₂/TFA (1:1, 2 mL) was stirred at room temperature for 90 minutes. The solvent was removed *in vacuo* to give a transparent oil. This oil was added to a stirring solution of benzylsulfonyl-D-serine-L-alanine carboxylate (63 mg, 0.194 mmol), HATU (147 mg, 0.387 mmol), HOAT (52 mg, 0.387 mmol), and DIEA (135 μL, 0.774 mmol) in acetonitrile (1.0 mL). The mixture was stirred overnight at room temperature. HPLC purification (CH₃CN, H₂O, 0.1% TFA) gave a fluffy white solid as the title compound (15 mg, 16%), MS (electrospray) 478 (M + 1).

Example AIn vitro enzyme Assays for specificity determination

The ability of compounds of the present invention to act as selective inhibitors of urokinase catalytic activity was assessed by determining the concentration of test compound which inhibited the activity of this enzyme by 50%, (IC_{50}), and comparing this value to that determined for all or some of the following related serine proteases: recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, factor Xa, thrombin and trypsin.

The buffer used for all assays was HBSA (10mM HEPES, pH 7.5, 150mM sodium chloride, 0.1% bovine serum albumin).

The assay for IC_{50} determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration (covering a broad concentration range) diluted in HBSA (or HBSA alone for V_0 (uninhibited velocity) measurement), and 50 microliters of the enzyme diluted in HBSA. Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate at the concentrations specified below were added to the wells, yielding a final total volume of 200 microliters (about 4 times K_m). The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value. K_i may be calculated from the IC_{50} value.

Urokinase Assay

Urokinase catalytic activity was determined using the chromogenic substrate 150mM S-2444 (L-Pyroglutamyl-glycyl-L-arginine-p-nitroaniline hydrochloride), obtained from 5 DiaPharma Group, Inc. Urokinase (Abbokinase), manufactured by Abbott Laboratories, was obtained from Priority Pharmaceuticals and diluted to 750pM in the HBSA assay buffer prior to use. The assay buffer was HBS (10mM HEPES, 150mM sodium chloride pH 7.4) with 0.1% BSA. K_i was 10 calculated using the IC₅₀ value.

Thrombin (fIIa) Assay

Enzyme activity was determined using the chromogenic substrate, Pefachrome t-PA (CH_3SO_2 -D-hexahydrotyrosine-glycyl-L-Arginine-p-nitroaniline, obtained from Pentapharm 15 Ltd.). The substrate was reconstituted in deionized water prior to use. Purified human α -thrombin was obtained from Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150mM sodium chloride, 0.1% bovine serum albumin).

IC₅₀ determinations were conducted where HBSA (50 μL), α -thrombin (50 μl) (the final enzyme concentration is 0.5nM) and inhibitor (50 μl) (covering a broad concentration range), were combined in appropriate wells and incubated for 30 minutes at room temperature prior to the addition of 25 substrate Pefachrome-t-PA (50 μl) (the final substrate concentration is 250 μM , about 5 times Km). The initial velocity of Pefachrome t-PA hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 30 5% of the added substrate was utilized. The concentration

of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC₅₀ value.

Factor Xa

Factor Xa catalytic activity was determined using the 5 chromogenic substrate S-2765 (N-benzyloxycarbonyl-D-arginine-L-glycine-L-arginine-p-nitroaniline), obtained from DiaPharma Group (Franklin, OH). All substrates were reconstituted in deionized water prior to use. The final concentration of S-2765 was 250µM (about 5-times Km).
10 Purified human Factor X was obtained from Enzyme Research Laboratories, Inc. (South Bend, IN) and Factor Xa (FXa) was activated and prepared from it as described [Bock, P.E., Craig, P.A., Olson, S.T., and Singh, P., Arch. Biochem. Biophys. 273:375-388 (1989)]. The enzyme was diluted into
15 HBSA prior to assay in which the final concentration was 0.25nM.

Recombinant tissue plasminogen activator (rt-PA) Assay

rt-PA catalytic activity was determined using the substrate, Pefachrome t-PA (CH₃SO₂-D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 500 micromolar (about 3-times Km). Human rt-PA (Activase®) was obtained from Genentech Inc.
20
25 The enzyme was reconstituted in deionized water and diluted into HBSA prior to the assay in which the final concentration was 1.0nM.

Plasmin Assay

Plasmin catalytic activity was determined using the chromogenic substrate, S-2366 [L-pyroglutamyl-L-prolyl-L-arginine-p-nitroaniline hydrochloride], which was obtained from DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 2.5-times Km). Purified human plasmin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0nM.

Activated Protein C (aPC) Assay

aPC catalytic activity was determined using the chromogenic substrate, Pefachrome PC (delta-carbobenzyloxy-D-lysine-L-prolyl-L-arginine-p-nitroaniline dihydrochloride), obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 400 micromolar (about 3 times Km). Purified human aPC was obtained from Hematologic Technologies, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0nM.

Chymotrypsin Assay

Chymotrypsin catalytic activity was determined using the chromogenic substrate, S-2586 (methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide), which was obtained from DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 100 micromolar (about 9-times Km). Purified (3X-crystallized; CDI) bovine

pancreatic α -chymotrypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5nM.

5 Trypsin Assay

Trypsin catalytic activity was determined using the chromogenic substrate, S-2222 (benzoyl-L-isoleucine-L-glutamic acid-[gamma-methyl ester]-L-arginine-p-nitroanilide), which was obtained from DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 4-times Km). Purified (3X-crystallized; TRL3) bovine pancreatic trypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5nM.

Tables IA and IB

Tables IA and IB list the determined K_i or IC_{50} values for certain of the enzymes listed above for compounds of the present invention that demonstrate a high degree of specificity for the inhibition of urokinase compared to other serine proteases.

TABLE IA

Compound (Figure 10A to 10F)	K_i uPA*	IC_{50} tPA*	IC_{50} Plasmin*
A	A	D	D
B	C	D	E

Compound (Figure 10A to 10F)	Ki uPA*	IC50 tPA*	IC50 Plasmin*
C	C	E	D
D	B	D	D
E	A	E	NT
F	A	E	NT
G	B	D	E
H	B	D	D
I	A	D	D
J	A	D	C
K	A	D	C
L	A	D	D
M	B	E	E
N	A	D	D
O	A	D	D
P	A	E	D
Q	A	E	D
R	A	E	D
S	B	E	E
T	A	D	C
U	A	C	C
V	A	D	C
W	B	E	E
X	A	D	D
Y	A	D	D
Z	A	E	D
AA	A	E	D
AB	A	E	D

Compound (Figure 10A to 10F)	Ki uPA*	IC50 tPA*	IC50 Plasmin*
AC	B	E	E
AD	A	D	C
AE	A	C	C
AF	A	D	C
AG	A	E	E
AH	A	C	D
AI	B	E	E
AJ	A	D	D
AK	A	D	D
AL	A	D	D
AM	A	E	E
AN	A	E	E
AO	B	E	E
AP	A	E	D
AQ	A	E	D
AR	C	E	E
AS	A	E	D
AT	A	E	D
AU	C	E	E
AV	A	E	E
AW	C	E	E

TABLE IB

Compound (Figure 13A to 13C)	Ki uPA*	Ki Plasmin*
BA	A	B
BB	A	E

BC	A	E
BD	A	C
BE	A	C
BF	A	E
BG	A	E
BH	A	E
BI	A	E
BJ	A	E
BK	A	E
BL	A	E
BM	A	E
BN	A	E
BO	A	E
BP	A	E
BQ	A	E

*

A = less than 100nM

B = 100-250nM

5 C = 250-2500nM

D = greater than 2500nM

E = Not Active

NT = Not tested

Example B10 Evaluation of Test Compound as an Inhibitor of AngiogenesisIn Vivo

The chicken CAM (chick embryo chorioallantoic membrane) model, a standard angiogenesis assay, is used to evaluate the ability of a test compound to inhibit angiogenesis.

15 This model is an established model for evaluation of activity of a test compound to affect formulation of new blood vessels.

A filter disc saturated with a 0.5 µg/ml solution of basic fibroblast growth factor (bFGF) is placed on the CAM of 10 day old chick embryos to induce angiogenesis. Twenty four hours later, 0 to 1 µg of Test Compound, in a total 5 volume of 100 µl of sterile PBS, is injected intravenously into the embryo. Approximately 48 hours later, the embryos are sacrificed and the filter discs and surrounding CAM tissue are excised for analysis. Angiogenesis is quantitated by counting the number of blood vessel branch 10 points within the confined region of the filter [Brooks, P.C., et al, Methods in Molecular Biology 120:257-269 (1999)]. The angiogenic index is defined as the difference in the number of blood vessel branch points between an experimental group and the untreated, control embryos. Each 15 experimental group will contain 8 to 10 chicken embryos.

Example C

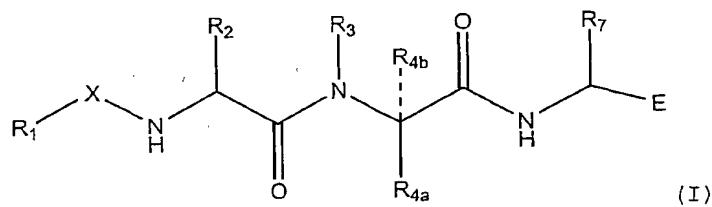
Evaluation of Test Compound to Inhibit the Growth of Human Tumor Cells in a Chick Embryo Model

A chicken embryo model is used to evaluate the activity 20 of Test Compound to inhibit the growth of human tumor cells *in vivo*. A single cell suspension of human fibrosarcoma cells (HT 1080), containing 4×10^5 cells in a total volume of 40 µl, is applied to 10 day old chick embryos as described by Brooks, et al ("Brooks, P.C., et al, "Integrin α_vβ₃ Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels", Cell 79:1157-1164 25 (1994)). Twenty-four hours later 0 to 10 µg of Test Compound are injected intravenously into the embryos. Following this single administration of compound, control 30 and treated embryos are incubated for a total of 7 days and

then sacrificed. Tumors are excised, trimmed free of surrounding CAM tissue, and weighed. The wet weights for tumors excised in this experiment are tabulated. Each experimental group will contain 10 to 12 chicken embryos.

We claim:

1. A compound of the formula:



5

wherein:

(a) X is selected from the group consisting of $-S(O)_2^-$, $-N(R')-S(O)_2^-$, $-(C=O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R')-$, and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with the proviso that when X is $-P(O)(R')-$, then R' is not hydrogen;

(b) R_1 is selected from the group consisting of:
 (1) alkyl of 1 to about 12 carbon atoms which is unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of Y_1 and Y_2 ,
 (2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 3 to about 8 carbon atoms which is unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of Y_1 , Y_2 , and Y_3 ,
 (3) cycloalkyl of 3 to about 15 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y_1 , Y_2 , and Y_3 ,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including, -N(_v) wherein -N(_v) is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂- , -O- , -S(=O)- , -S(O)₂- or -S- , which is unsubstituted or mono-, di-, or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(6) alkenyl of 2 to about 6 carbon atoms which is unsubstituted or substituted with cycloalkyl of about 3 to about 8 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(7) aryl of about 6 to about 14 carbon atoms which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-

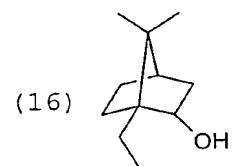
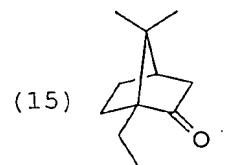
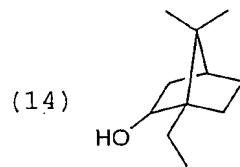
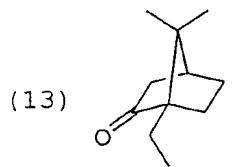
substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(9) aralkyl of about 7 to about 15 carbon atoms which is unsubstituted or substituted on the alkyl chain 5 with hydroxy or halogen and which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(10) heteroaralkyl of about 5 to about 14 ring 10 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted on the ring or 15 mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(11) aralkenyl of about 8 to about 16 carbon atoms which is unsubstituted or mono-, di-, or tri-substituted on 20 the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from 25 oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,



5 (17) fused carbocyclic alkyl of about 9 to about
15 carbon atoms,

(18) difluoromethyl or perfluoroalkyl of 1 to
about 12 carbon atoms,

10 (19) perfluoroaryl of about 6 to about 14 carbon
atoms,

(20) perfluoroaralkyl of about 7 to about 15
carbon atoms, and

15 (21) hydrogen when X is a direct link;
wherein each Y₁, Y₂, and Y₃ is independently selected and is
(i) selected from the group consisting of
halogen, cyano, nitro, tetrazolyl, guanidino, amidino,
methylguanidino, -CF₃, -CF₂CF₃, -CH(CF₃)₂, -C(OH)(CF₃)₂, -OCF₃,
-OCF₂H, -OCF₂CF₃, -OC(O)NH₂, -OC(O)NZ₁, -OC(O)NZ₁Z₂,
-NHC(O)Z₁, -NHC(O)NH₂, -NHC(O)NZ₁, -NHC(O)NZ₁Z₂, -C(O)OH,
20 -C(O)OZ₁, -C(O)NH₂, -C(O)NZ₁, -C(O)NZ₁Z₂, -P(O)₃H₂,

-P(O)₃(Z₁)₂, -S(O)₃H, -S(O)_mZ₁, -Z₁, -OZ₁, -OH, -NH₂, -NHz₁, -NZ₁Z₂, -C(=NH)NH₂, -C(=NOH)NH₂, -N-morpholino, and -S(O)_m(CF₂)_qCF₃, wherein m is 0, 1 or 2, q is an integer from 0 to 5, and Z₁ and Z₂ are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms, or

10 (ii) Y₁ and Y₂ are selected together to be -O[C(Z₃)(Z₄)]_rO- or -O[C(Z₃)(Z₄)]_{r+1}-, wherein r is an integer from 1 to 4 and Z₃ and Z₄ are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms;

15 (c) R₂ is selected from the group consisting of -CH₃, -C₂H₅, -(CH₂)₂OH, -(CH₂)₂OA₁, -CH(R₅)OH, -CH(R₅)OA₁ and -CH₂NH-X'-R₆ wherein A₁ is -C(=O)OR₆, -C(=O)R₆ or -C(=O)NR₅R₆; 20 X' is selected from the group consisting of -S(O)₂-, -S(O)₂-N(R'')-, -(C=O)-, -C(=O)-O-, -C(=O)-NH-, -P(O)(R'')-, and a direct link, wherein R'' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms with the proviso that when X' is -P(O)(R'')-, then R'' is not hydrogen; R₅ is selected from the group consisting of:

25 (1) alkyl of 1 to about 4 carbon atoms, which is unsubstituted or substituted with 1 to 2 substituents
30 selected from the group consisting of Y₁ and Y₂,
(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 6 carbon atoms,

which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(3) cycloalkyl of 3 to about 6 carbon atoms,
5 which is unsubstituted or mono-, di-, or trisubstituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(4) heterocycloalkyl of 4 to about 6 ring atoms
with the ring atoms selected from carbon and heteroatoms,
10 wherein the heteroatoms are selected from the group
consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1
or 2, which is unsubstituted or mono-, di-, or tri-
substituted on the ring with 1 to 3 substituents selected
from the group consisting of Y₁, Y₂, and Y₃,

15 (5) heterocyclo of 4 to about 6 ring atoms with
the ring atoms selected from carbon and heteroatoms, wherein
the heteroatoms are selected from the group consisting of
oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2,
including -N(_v), wherein -N(_v) is a 5 to 7 member

20 heterocycle having 3 to 6 ring carbon atoms, where V is
-CH₂- , -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted
or mono-, di-, or tri-substituted on the ring carbons with 1
to 3 substituents selected from the group consisting of Y₁,
Y₂, and Y₃,

25 (6) alkenyl of 2 to about 6 carbon atoms which is
unsubstituted or substituted with cycloalkyl of 3 to about 6
carbon atoms, which is unsubstituted or mono-, di-, or tri-
substituted on the ring with 1 to 3 substituents selected
from the group consisting of Y₁, Y₂, and Y₃,

(7) phenyl which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

5 (8) heteroaryl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

10 (9) alkyl of 1 to about 4 carbon atoms substituted with phenyl and which is unsubstituted or mono-, di-, or tri-substituted on the phenyl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

15 (10) heteroaralkyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and 20 which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(11) aralkenyl of about 8 to about 12 carbon atoms which is unsubstituted or mono-, di-, or tri-substituted on 25 the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

(12) heteroaralkenyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from 30 oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted on the ring carbons with 1 to

3 substituents selected from the group consisting of Y₁, Y₂, and Y₃, and

(13) hydrogen; and

R₆ is selected from the group consisting of:

5 (1) alkyl of 1 to about 12 carbon atoms, which is unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of Y₁, and Y₂,

10 (2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 8 carbon atoms, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

15 (3) cycloalkyl of 3 to about 15 carbon atoms, which is unsubstituted or mono-, di-, or trisubstituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

20 (4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is unsubstituted or mono-, di-, or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

25 (5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including -N(_v), wherein -N(_v) is a 5 to 7 member

30 heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is unsubstituted

or mono-, di-, or tri-substituted on the ring carbons with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

5 (6) aryl of about 6 to about 14 carbon atoms which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

10 (7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or mono-, di- or tri-substituted with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

15 (8) aralkyl of about 7 to about 15 carbon atoms which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and which is unsubstituted or mono-, di-, or tri-substituted on the aryl ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃,

20 (9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is unsubstituted or substituted on the alkyl chain with hydroxy or halogen and 25 which is unsubstituted on the ring or mono-, di- or tri-substituted on the ring with 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃, and

(10) hydrogen, with the proviso that R₆ is not hydrogen when A₁ is -C(=O)OR₆;

30 (d) R₃ is selected from H or methyl, or R₃, and R_{4a} and R_{4b} are selected together as set forth in (f);

(e) (i) R_{4a} is in the S configuration and is selected from the group consisting of H, $-CH_2-S-CH_3$, $-CH_2OH$, $-CH_2CN$, lower alkyl of 1 to about 3 carbon atoms, $-CH_2C\equiv CH$, $-CH_2CH=CH_2$ and $-CH=CH_2$ and R_{4b} is hydrogen;

5 (ii) R_{4a} and R_{4b} are independently lower alkyl of 1 to 3 carbon atoms;

(iii) R_{4a} and R_{4b} are selected together and are $-(CH_2)_k-$ wherein k is 5 or 6 to give a spirocycloalkyl group; or

10 (iv) R_3 , and R_{4a} and R_{4b} are selected together as set forth in (f);

(f) alternatively, R_3 and R_{4a} are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecolyl, 15 azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 4-aminoprolyl, 4- $(-CH_2NH_2)$ -prolyl, 3,4-methanoprolyl and 3,4-dehydropopylyl and R_{4b} is hydrogen;

(g) R_7 is hydrogen or alkyl of 1 to about 4 carbon atoms; and

20 (h) E is Q-T

wherein (i) Q is selected from the group consisting of $-C(R_{13}R_{14})_t-$, phenyl substituted with R_8 and R_9 , a 5- or 6-membered heterocyclic ring having 1 to 2 heteroatoms substituted with R_8 or R_9 and R_9 , and a 9- or 10-membered 25 heterocyclic ring having 1 to 2 heteroatoms substituted with R_8 and R_9 , wherein heteroatoms are selected from nitrogen and sulfur; and

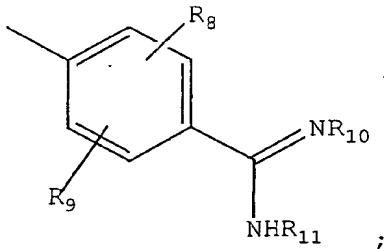
(ii) T is selected from the group consisting of $-C(=NR_{10})NHR_{11}$, $-NH-C(=NR_{10})NHR_{11}$ and $-NHR_{15}$;

30 wherein R_8 and R_9 are independently selected from the group consisting of hydrogen, hydroxy, halogen, alkyl of 1 to about 4 carbon atoms, alkyl of 1 to about 4 carbon atoms

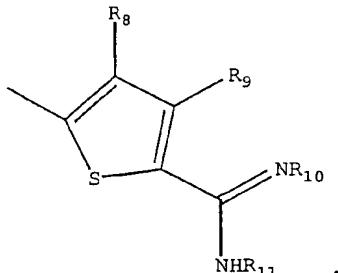
substituted with alkoxy of 1 to about 4 carbon atoms, alkoxy of 1 to about 6 carbon atoms, and trifluoromethyl; R₁₀ and R₁₁ are independently hydrogen, hydroxy, alkoxy of 1 to about 3 carbon atoms, trihydrocarbylsilyl of 3 to about 16 carbon atoms, alkyl of 1 to about 3 carbon atoms or -C(=O)R₁₂ with the proviso that R₁₀ and R₁₁ are not both hydroxy or alkoxy; R₁₂ is hydrogen, alkyl of 1 to about 6 carbon atoms, alkoxy of 1 to about 6 carbon atoms or (CF₂)_jCF₃ wherein j is 0, 1, 2 or 3; each of R₁₃ and R₁₄ is independently selected from the group consisting of hydrogen and lower alkyl of 1 to about 3 carbon atoms; R₁₅ is selected from the group consisting of hydrogen, alkyl of 1 to about 6 carbon atoms and -(CF₂)_hCF₃ wherein h is 0, 1, 2 or 3 and t is an integer from 0 to 6; and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1 wherein E is selected from the group consisting of

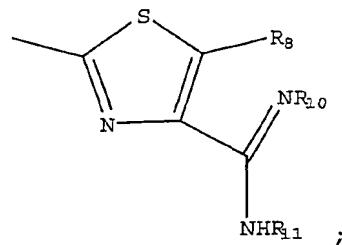
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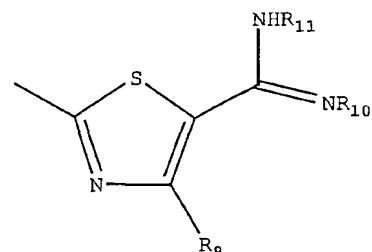
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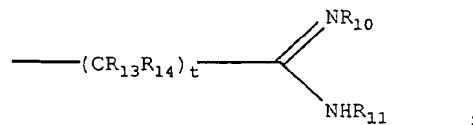


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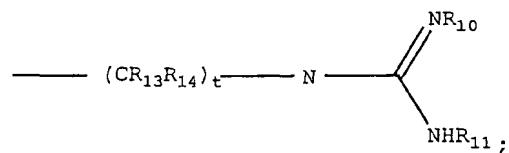


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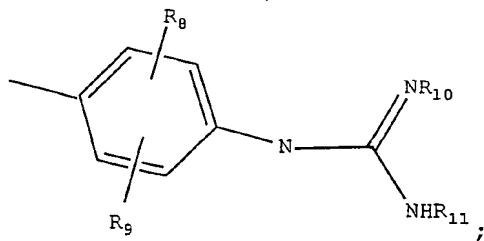


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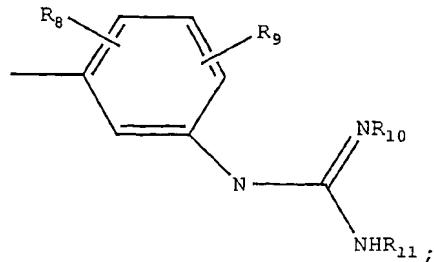


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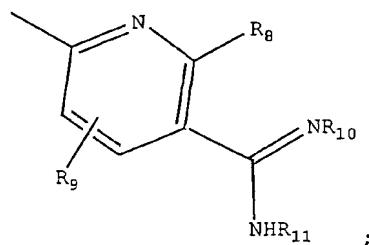
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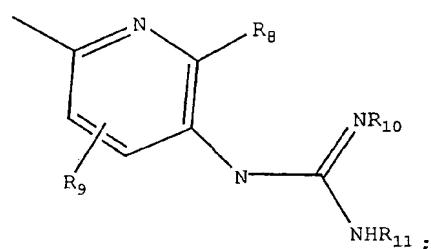


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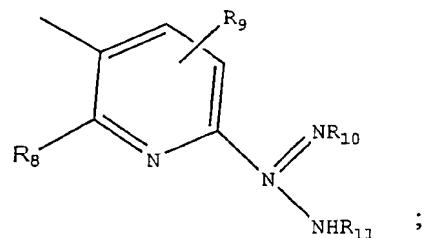


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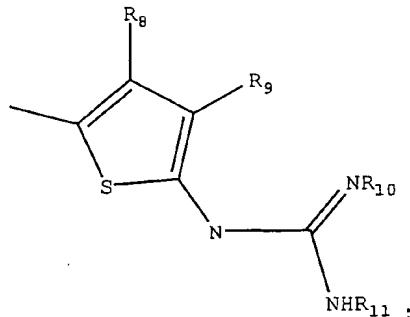


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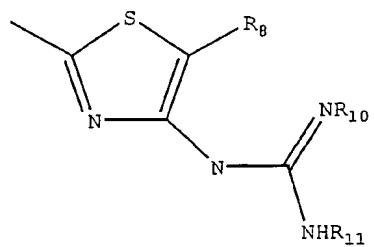


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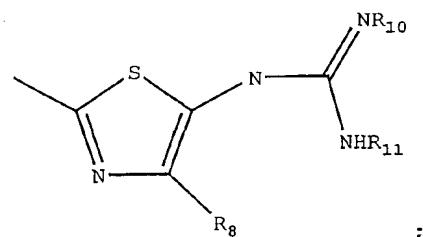


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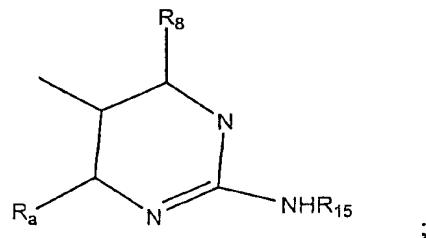


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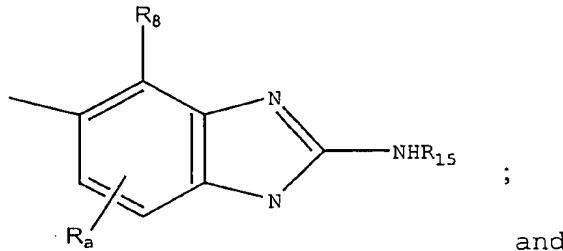
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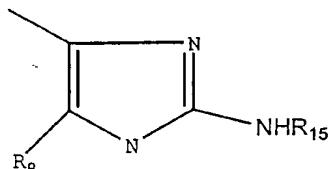
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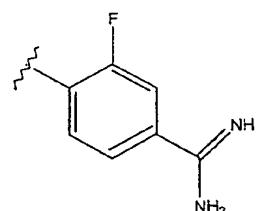
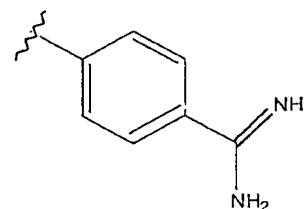


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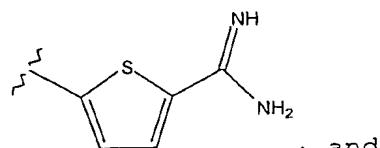


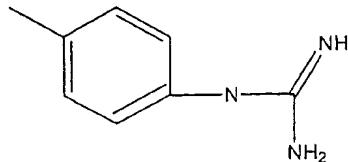
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3. A compound according to claim 1 wherein E is selected from the group consisting of



10





4. A compound according to claim 3 wherein X is -S(O)₂- or -O-C(=O)-.

5. A compound according to claim 4 wherein R₂ is -CH₃ or -CH(R₅)OH.

6. A compound according to claim 5 wherein R₅ is hydrogen, unsubstituted alkyl or alkyl substituted with Y₁.

7. A compound according to claim 6 wherein R₂ is -CH₂OH or -CH(CH₃)OH.

10 8. A compound according to claim 7 wherein R₂ is -CH(CH₃)OH and has the R configuration.

9. A compound according to claim 7 wherein R₃ is hydrogen.

10 15 10. A compound according to claim 9 wherein R₄ is methyl or propargyl and R_{4b} is hydrogen.

11. A compound according to claim 7 wherein R₃ and R_{4a} are selected together to give a group at P₂ selected from the group consisting of prolyl, pipecolyl, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 3,4-methanoprolyl and 3, 20 4-dehydropopylyl and R_{4b} is hydrogen.

12. A compound according to claim 11 wherein R₃ and R_{4a} are selected together to give a group at P₂ selected from the group consisting of prolyl, 4-cis-hydroxyprolyl, 3,4-

dehydroprolyl, 3,4-methanoprolyl and azetidine-2-carbonyl and R_{4b} is hydrogen.

13. A compound according to claim 5 wherein R₅ is H.

14. A compound according to claim 3 wherein X is
5 selected from the group consisting of -S(O)₂- , -OC(=O)- , -NH-C(=O)- and a direct link.

15. A compound according to claim 14 wherein X is
-S(O)₂- or -OC(=O)-.

16. A compound according to claim 15 wherein R₁ is
10 selected from the group consisting of phenyl, benzyl,
2-phenylethyl, isobutyl, n-butyl, 3-phenylpropyl, 4-
chlorobenzyl, 3-chlorobenzyl and 2-fluorobenzyl.

17. A compound according to claim 16 wherein R₁-X- is
selected from the group consisting of phenyl-S(O)₂- , benzyl-
15 S(O)₂- , 2-phenylethyl-S(O)₂- , 3-phenylpropyl-S(O)₂- ; n-butyl-
S(O)₂- , benzyl-OC(=O)- , isobutyl-OC(=O)- 4-chlorobenzyl-
S(O)₂- , 3-chlorobenzyl-S(O)₂- and 2-fluorobenzyl-S(O)₂- .

18. A compound according to claim 17 wherein R₂ is
selected to give a D-seryl group at P3.

20 19. A compound according to claim 18 wherein E is 4-
amidinophenyl, 4-guanidinophenyl or 5-(2-amidino-thienyl).

20. A compound according to claim 19 wherein (i) R₃ and
R_{4a} are selected together to give a group at P₂ selected from
the group consisting of prolyl, azetidine-2-carbonyl, 3,4-
25 methanoprolyl and 3,4-dehydroprolyl, or (ii) R₃ is hydrogen
and R_{4a} is methyl, and R_{4b} is hydrogen.

21. A compound according to claim 17 wherein E is 4-amidinophenyl, 4-guanidinophenyl or 5-(2-amidino-thienyl).

22. A compound according to claim 21 wherein (i) R₃ and R_{4a} are selected together to give a group at P₂ selected from 5 the group consisting of prolyl, azetidine-2-carbonyl, 3,4-methanoprollyl and 3,4-dehydroprolyl, or (ii) R₃ is hydrogen and R_{4a} is methyl, and R_{4b} is hydrogen.

23. A compound according to claim 17 wherein (i) R₃ and R_{4a} are selected together to give a group at P₂ selected from 10 the group consisting of prolyl, azetidine-2-carbonyl, 3,4-methanoprollyl and 3,4-dehydroprolyl, or (ii) R₃ is hydrogen and R_{4a} is methyl, and R_{4b} is hydrogen.

24. A compound according to claim 1 wherein R₂ is selected from the group consisting of -CH₂NH(X')(R₆) and 15 -CH(R₅)OH.

25. A compound according to claim 24 wherein R₅ is selected from the group consisting of hydrogen, unsubstituted alkyl, alkyl substituted with Y₁ and alkyl of 1 to 4 carbon atoms substituted with unsubstituted phenyl or 20 phenyl mono-, di- or tri-substituted 1 to 3 substituents selected from the group consisting of Y₁, Y₂, and Y₃.

26. A compound according to claim 25 wherein R₂ is selected to give a group at P₃ selected from the group consisting of D-seryl, (R,R)'D-allothreonyl, D-2-aminobutyryl, N-β-methyloxycarbonyl-D-2,3-diaminopropionyl, 25 N-β-(2-phenylethylcarbonyl)-D-2,3-diaminopropionyl, and N-β-benzylloxycarbonyl-D-2,3-diaminopropionyl.

27. A compound according to 26 wherein P3 is D-seryl or (R,R)D-allothreonyl.

28. A compound according to claim 1 wherein R₃ is hydrogen.

5 29. A compound according to claim 1 wherein R_{4a} is methyl, vinyl, allyl or propargyl and R_{4b} is hydrogen.

30. A compound according to claim 1 wherein R₃ and R_{4a} are selected together to give a group at P2 selected from the group consisting of prolyl, pipecolyl,
10 azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 3,4-methanoprollyl, and 3,4-dehydropoly, and R_{4b} is hydrogen.

31. A compound according to claim 30 wherein R₃ and R_{4a} are selected together to give a group at P2 selected from the group consisting of prolyl, 4-cis-hydroxyprolyl, 3,4-dehydropoly, 3,4-methanoprollyl and azetidine-2-carbonyl, and R_{4b} is hydrogen.

32. A compound according to claim 31 wherein R₂ is selected to give a group at P3 selected from the group consisting of D-seryl and (R,R)D-allothreonyl.

33. A compound according to claim 1 wherein R₂ is selected to give a group at P3 selected from the group consisting of D-seryl and (R₁R)-D-allothreonyl, R₃ is hydrogen, R_{4a} is methyl and R_{4b} is hydrogen.

25 34. A compound according to claim 1 wherein R₃ and R_{4a} are selected together to give a group at P2 selected from the group consisting of prolyl, azetidine-2-carbonyl, 3,4-methanoprollyl and 3,4-dehydropoly, and R_{4b} is hydrogen.

35. A compound according to claim 7 wherein R₂ is -CH₂OH and has the R configuration.

36. A compound according to claim 1 wherein R₂ is -(CH₂)₂OA₁ or -CH(R₅)OA₁.

5 37. A compound according to claim 36 wherein R₂ is -CH(R₅)OA₁.

38. A compound according to claim 37 wherein R₅ is selected from the group consisting of hydrogen, unsubstituted alkyl, alkyl substituted with Y₁, and alkyl of 10 1 to about 4 carbon atoms substituted with unsubstituted phenyl or phenyl mono-, di- or tri-substituted 1 to 3 substituents selected from the group consisting of Y₁, Y₂ and Y₃.

39. A compound according to claim 37 wherein R₂ is 15 selected to give a group at P3 selected from the group consisting of acyl and carbonate esters of D-seryl.

40. A compound according to claim 39 wherein R₃ is hydrogen.

41. A compound according to claim 40 wherein R_{4a} is 20 methyl, vinyl, allyl or propargyl and R_{4b} is hydrogen.

42. A compound according to claim 39 wherein R₃ and R₄ are selected together to give a group at P2 selected from the group consisting of prolyl, pipecolyl, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 25 3,4-methanoprolyl and 3,4-dehydropopylyl and R_{4b} is hydrogen.

43. A compound according to claim 1 wherein R₂ is selected to give a group at P3 selected from the group consisting of acyl and carbonate esters of D-seryl.

44. A compound according to claim 43 wherein R₃ is 5 hydrogen, R_{4a} is methyl and R_{4b} is hydrogen.

45. A compound according to claim 43 wherein R₃ and R_{4a} are selected together to give a group at P2 selected from the group consisting of prolyl, azetidine-2-carbonyl, 3,4-methanoprollyl and 3,4-dehydroprolyl, and R_{4b} is hydrogen.

10 46. A compound according to claim 1 wherein R₂ is selected to give a group at P3 selected from the group consisting of acyl and carbonate esters of D-seryl.

47. A compound according to claim 1 selected from the group consisting of the compounds depicted in Figures 10A to 15 10F, compound AX, compound AY and compound AZ.

48. A compound according to claim 1 selected from the group consisting of the compounds depicted in Figures 13A to 13C.

49. A compound according to claim 48 selected from the 20 group consisting of compound BG, compound BJ and compound BK.

50. The compound according to claim 48 which is compound BQ.

51. A compound according to claim 1 wherein Q is 25 phenyl substituted with R₈ and R₉ or pyridyl substituted with R₈ and R₉.

52. A compound according to claim 51 wherein T is

-C(=NR₁₀)NHR₁₁ or -NH-C(=NR₁₀)NHR₁₁.

53. A compound according to claim 52 wherein T is at the 4-position of Q.

54. A compound according to claim 53 wherein Q is phenyl substituted with R₈ and R₉.

55. A compound according to claim 54 wherein T is -NH-C(=NR₁₀)NHR₁₁.

56. A compound according to claim 53 wherein Q is pyridyl substituted with R₈ and R₉.

10 57. A compound according to claim 56 wherein T is -NH-C(=NR₁₀)NHR₁₁

58. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and therapeutically effective amount of compound of claim 1.

15 59. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 6.

20 60. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 17.

61. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 27.

25 62. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 30.

63. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 39.

64. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 41.

65. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 42.

10 66. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 44.

15 67. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 45.

68. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 47.

16 69. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 48.

70. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 49.

25 71. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises

administering to said mammal a therapeutically effective amount of a compound of claim 1.

72. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity
5 in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 6.

73. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity
10 in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 17.

74. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity
15 in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 27.

75. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity
20 in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 32.

76. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity
25 in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 39.

77. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective 5 amount of a compound of claim 41.

78. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective 10 amount of a compound of claim 42.

79. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective 15 amount of a compound of claim 44.

80. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective 20 amount of a compound of claim 45.

81. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective 25 amount of a compound of claim 47.

82. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises

administering to said mammal a therapeutically effective amount of a compound of claim 48.

83. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity
5 in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 49.

84. A method of reducing or inhibiting blood vessel formation in a mammal in need of such treatment which
10 comprises administering to said mammal a therapeutically effective amount of the compound of claim 47.

85. A method according to claim 84 wherein said blood vessel formation is related to a pathologic condition.

86. A method of reducing or inhibiting blood vessel formation in a mammal in need of such treatment which
15 comprises administering to said mammal a therapeutically effective amount of the compound of claim 48.

87. A method according to claim 86 wherein said blood vessel formation is related to a pathologic condition.

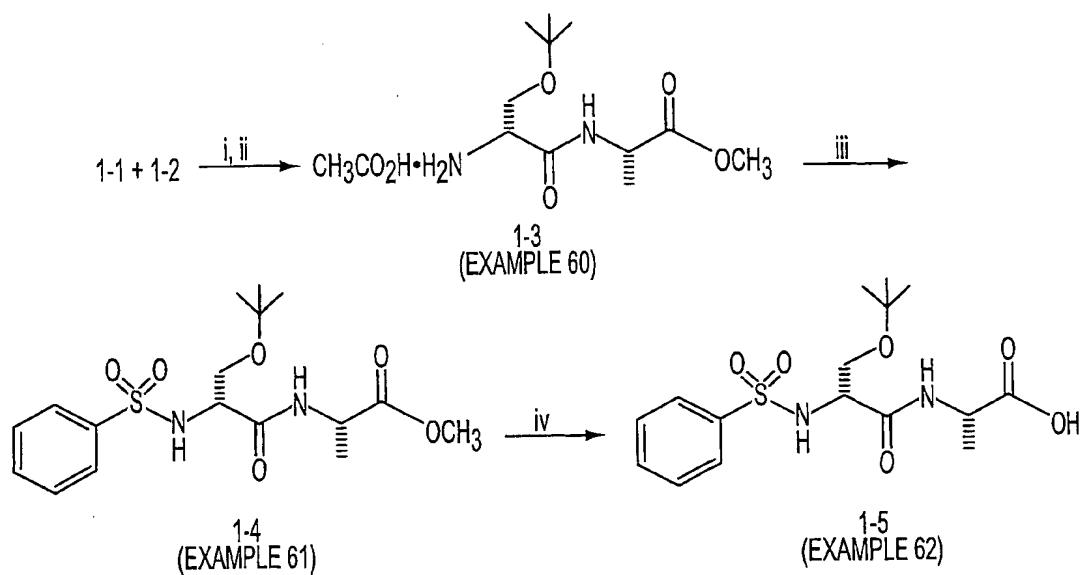


FIG. 1

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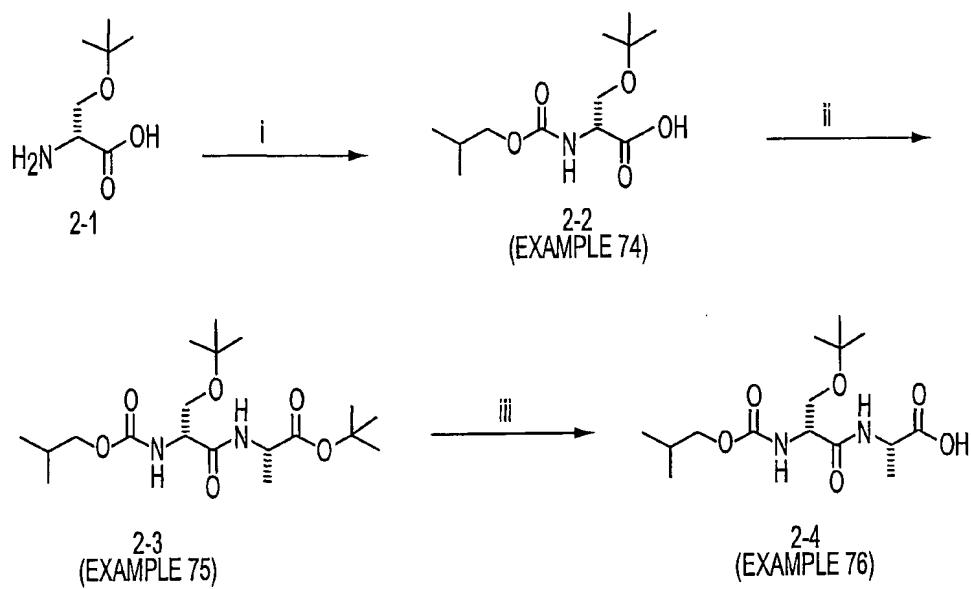


FIG. 2

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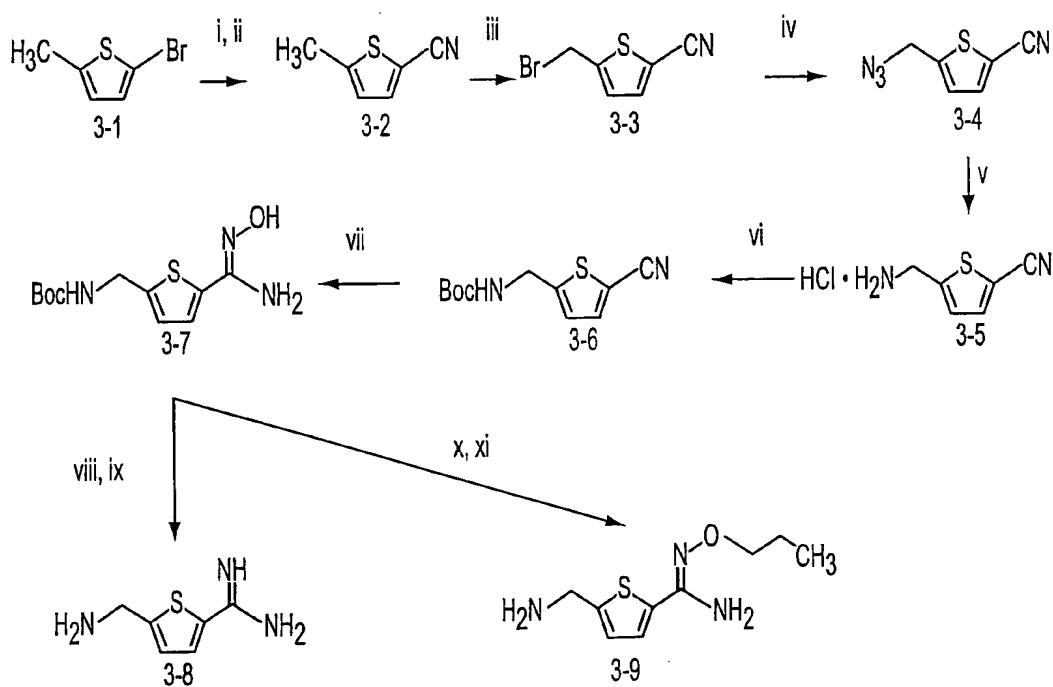


FIG. 3

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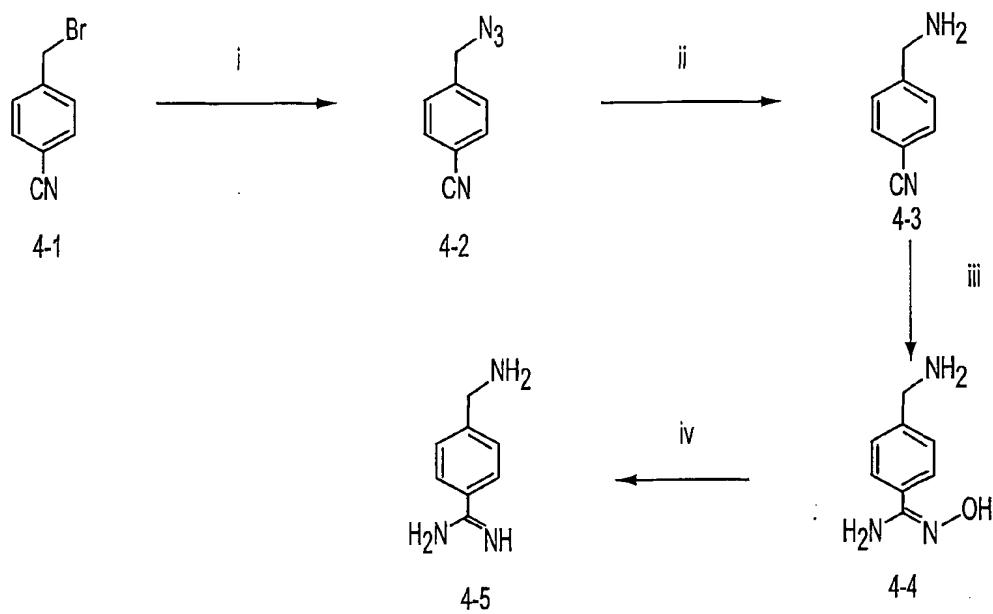


FIG. 4

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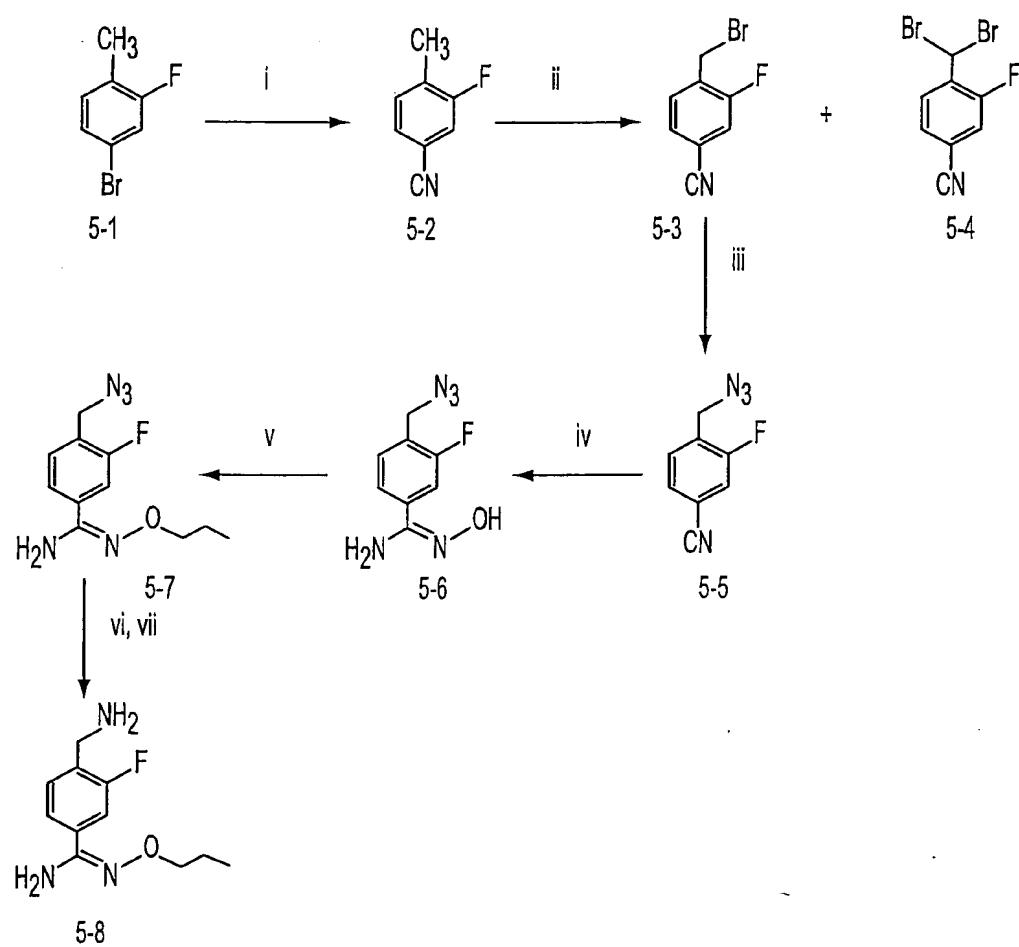


FIG. 5

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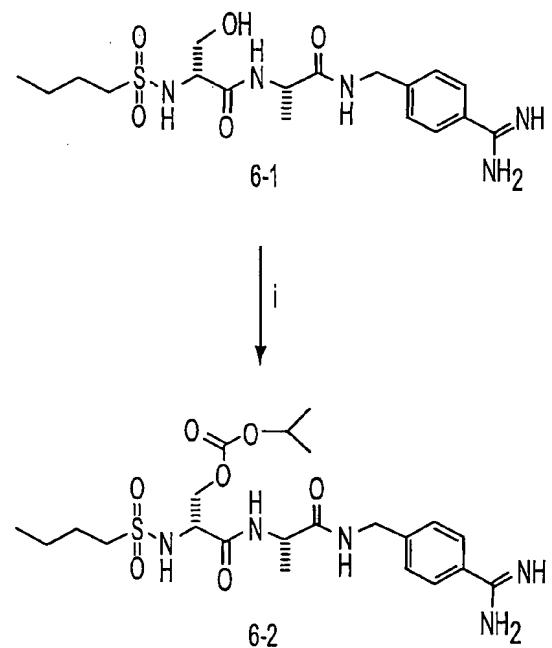


FIG. 6

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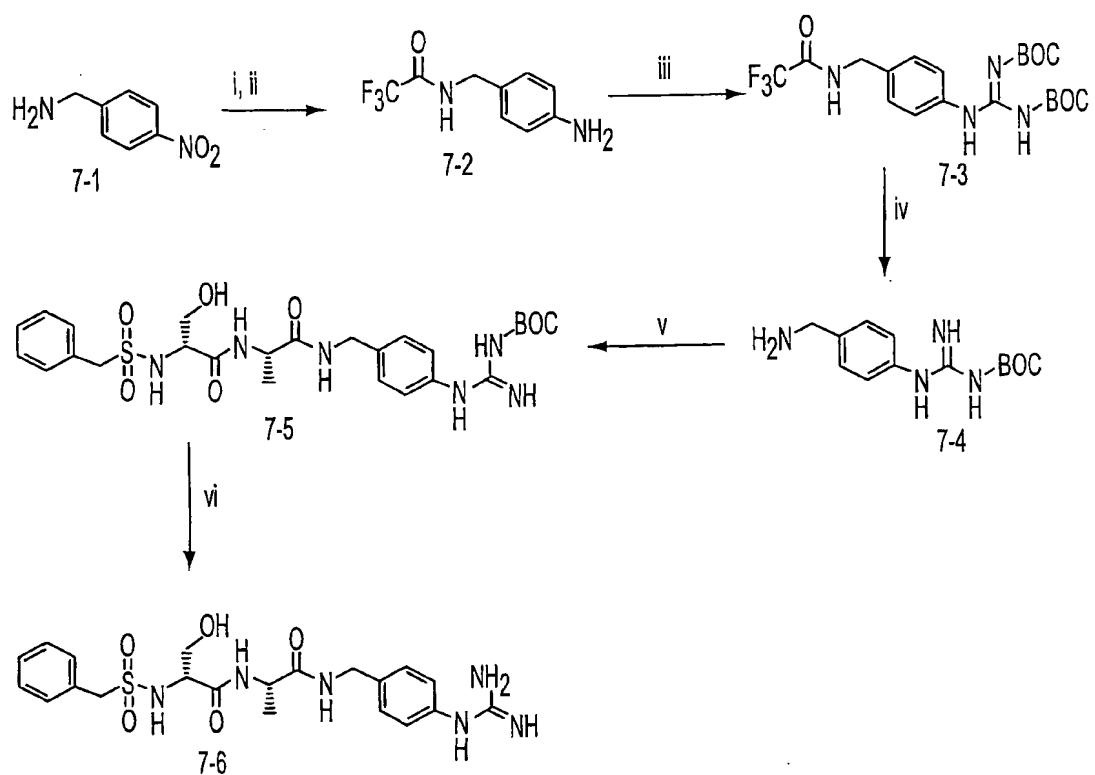


FIG. 7

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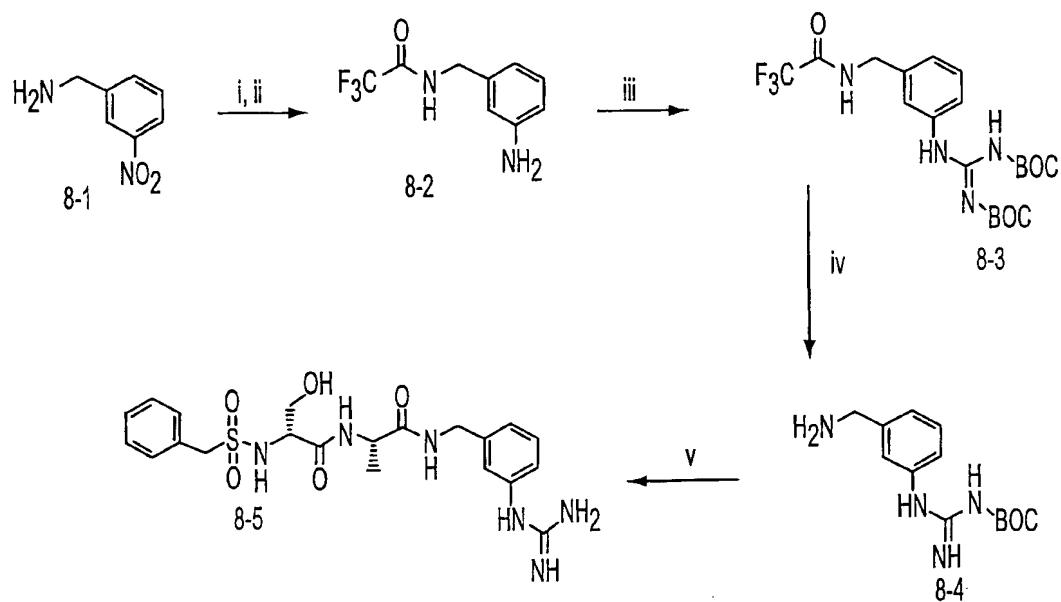


FIG. 8

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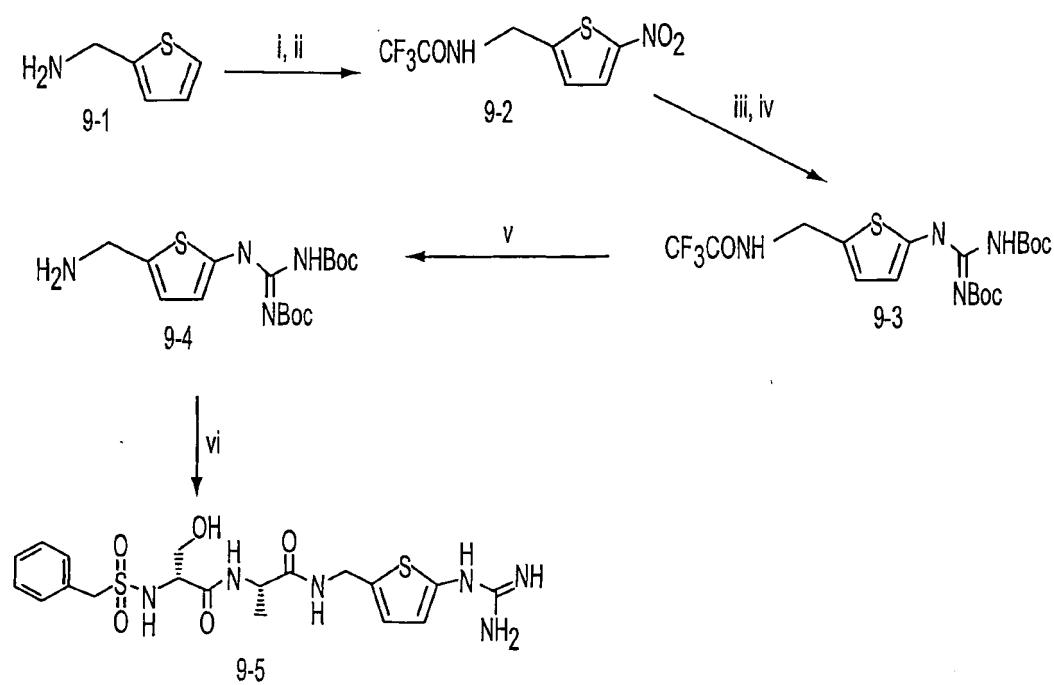


FIG. 9

SUBSTITUTE SHEET (RULE 26)

COMPOUND	STRUCTURE
A	
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J	

FIG. 10A

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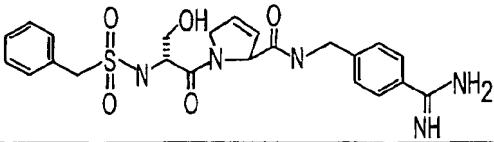
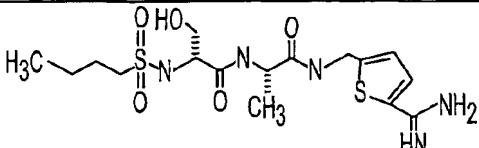
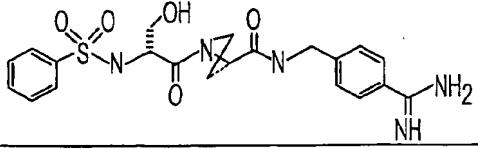
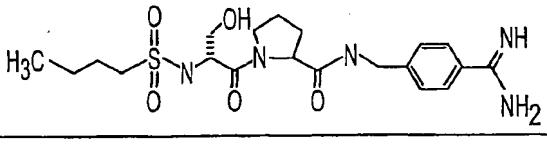
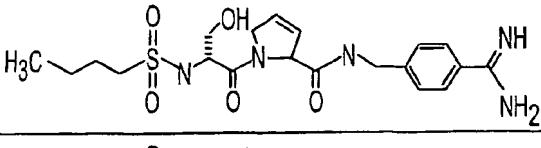
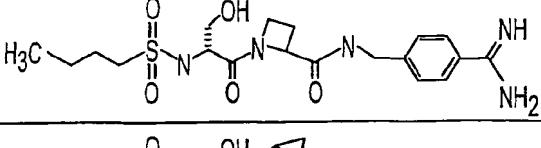
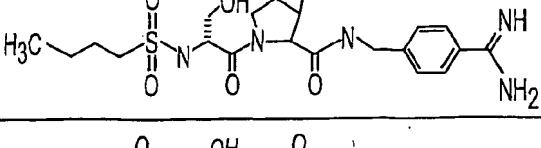
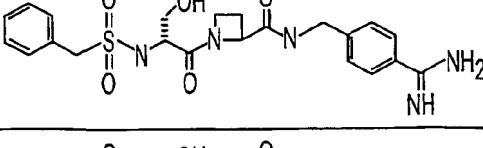
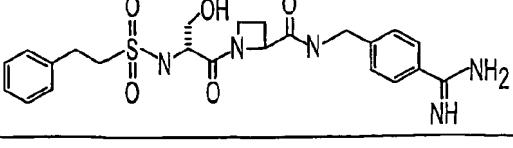
COMPOUND	STRUCTURE
K	
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O	
P	
Q	
R	
S	

FIG. 10B

SUBSTITUTE SHEET (RULE 26)

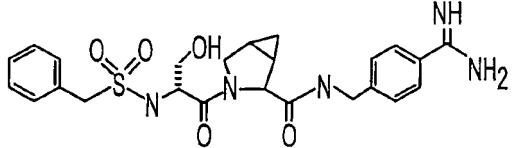
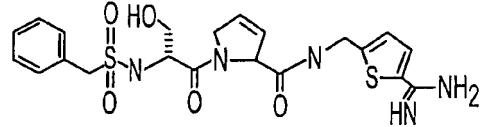
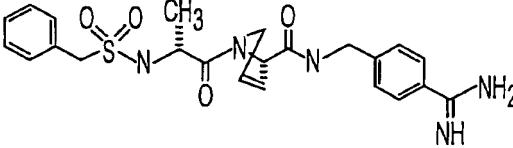
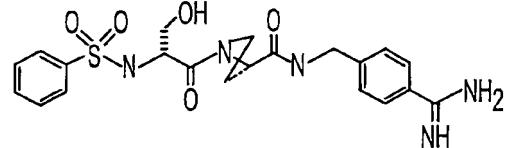
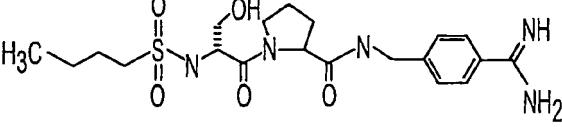
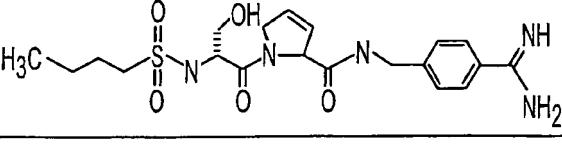
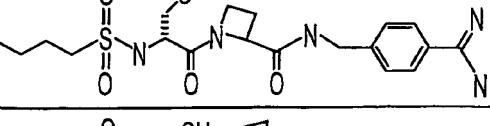
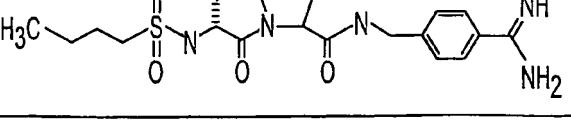
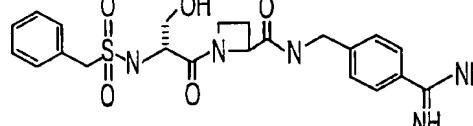
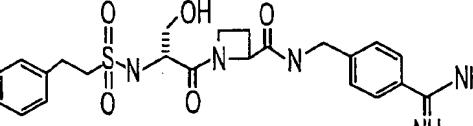
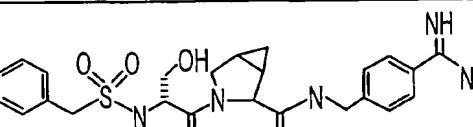
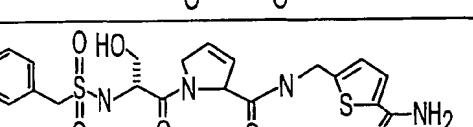
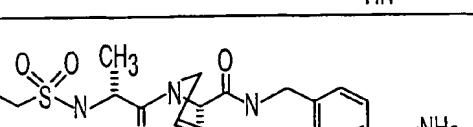
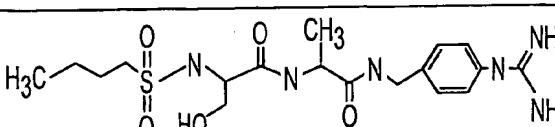
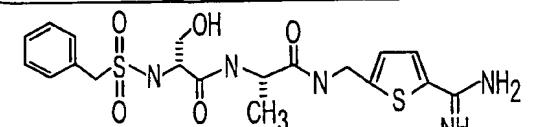
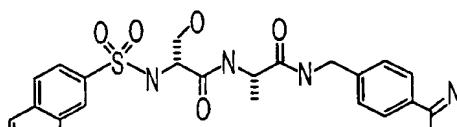
COMPOUND	STRUCTURE
T	
U	
V	
W	
X	
Y	
Z	
AA	

FIG. 10C

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FIG. 10D

COMPOUND	STRUCTURE
AB	
AC	
AD	
AE	
AF	
AG	
AH	
AI	

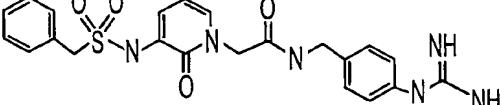
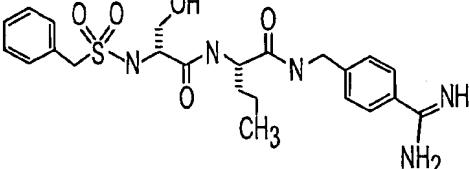
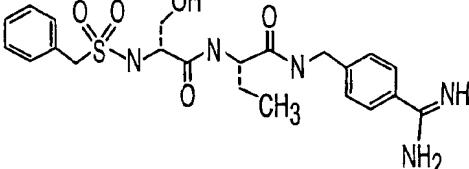
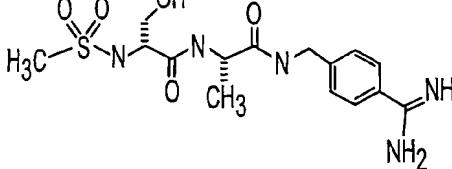
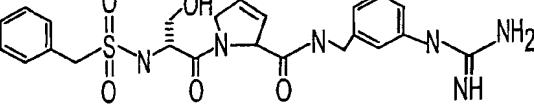
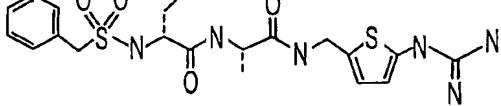
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COMPOUND	STRUCTURE
AJ	
AK	
AL	
AM	
AN	
AO	
AP	
AQ	

FIG. 10E

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FIG. 10F

COMPOUND	STRUCTURE
AR	
AS	
AT	
AU	
AV	
AW	

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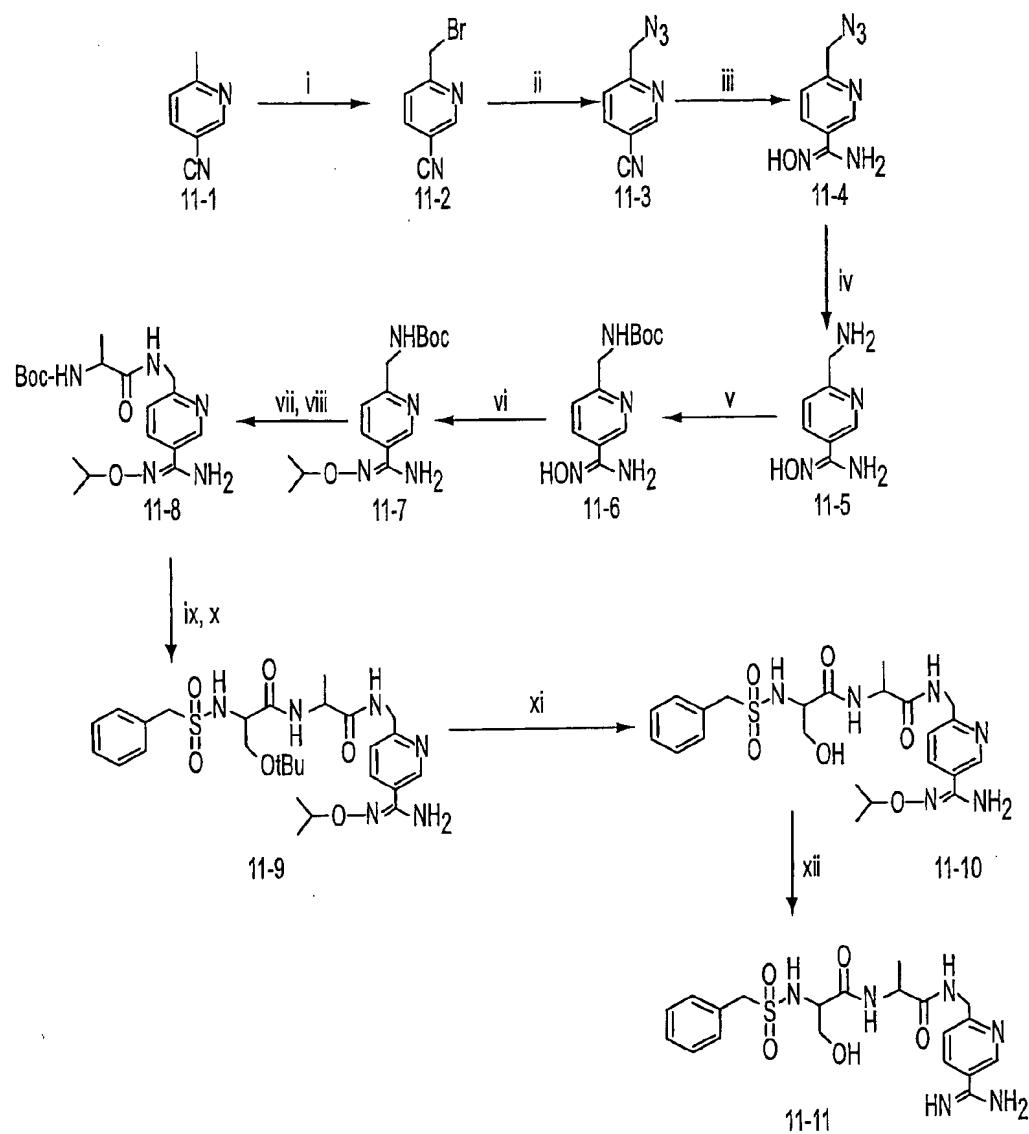


FIG. 11

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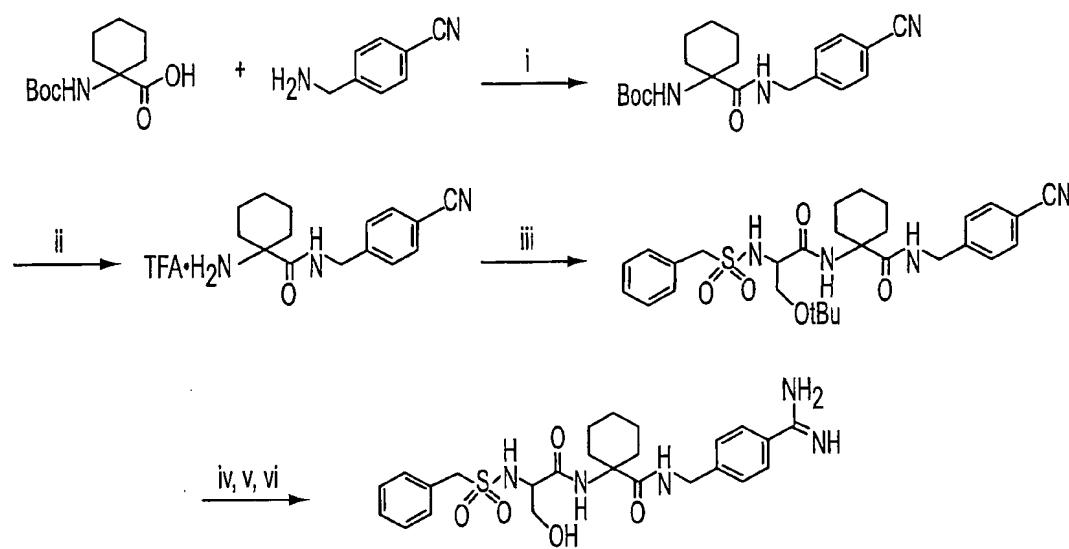


FIG. 12

SUBSTITUTE SHEET (RULE 26)

COMPOUND	STRUCTURE
BA	
BB	
BC	
BD	
BE	

FIG. 13A

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COMPOUND	STRUCTURE
BF	
BG	
BH	
BI	
BJ	
BK	

FIG. 13B

SUBSTITUTE SHEET (RULE 26)

COMPOUND	STRUCTURE
BL	
BM	
BN	
BO	
BP	
BQ	

FIG. 13C

SUBSTITUTE SHEET (RULE 26)

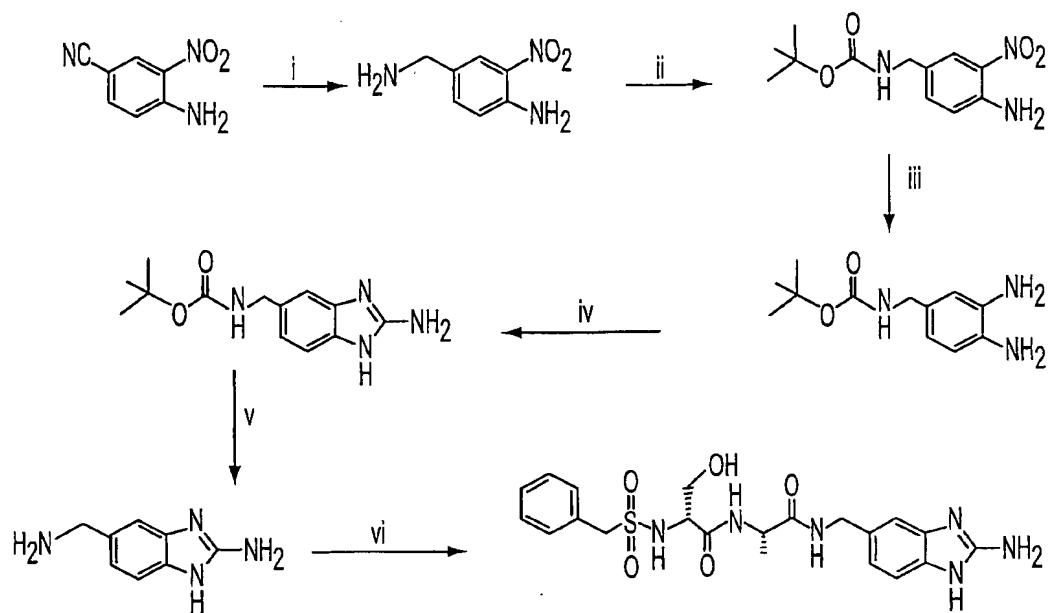


FIG. 14

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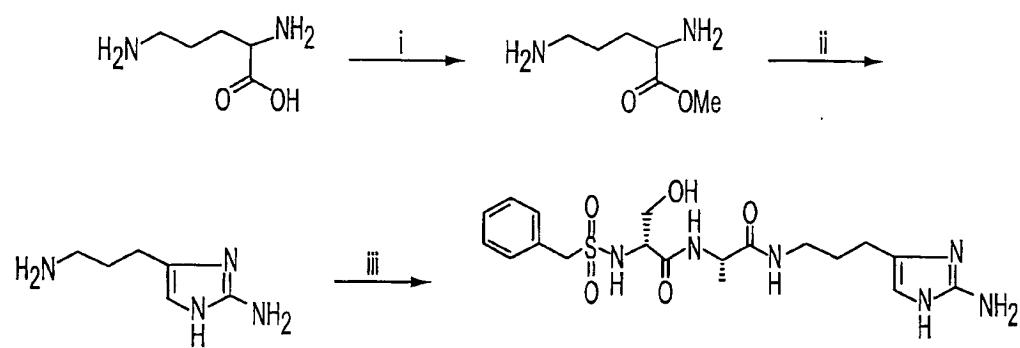


FIG. 15

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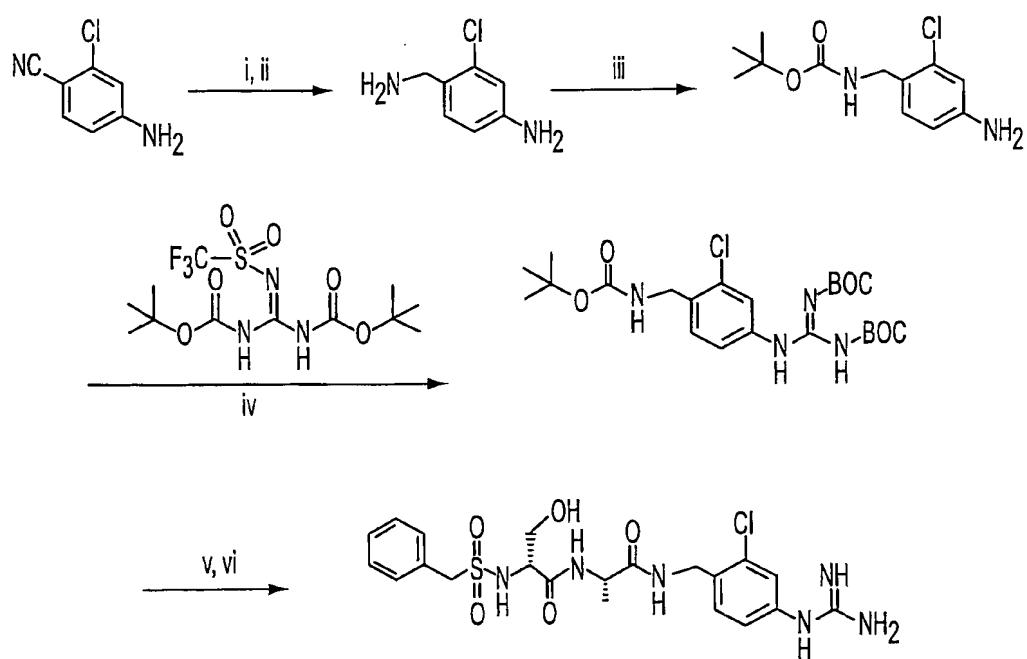


FIG. 16

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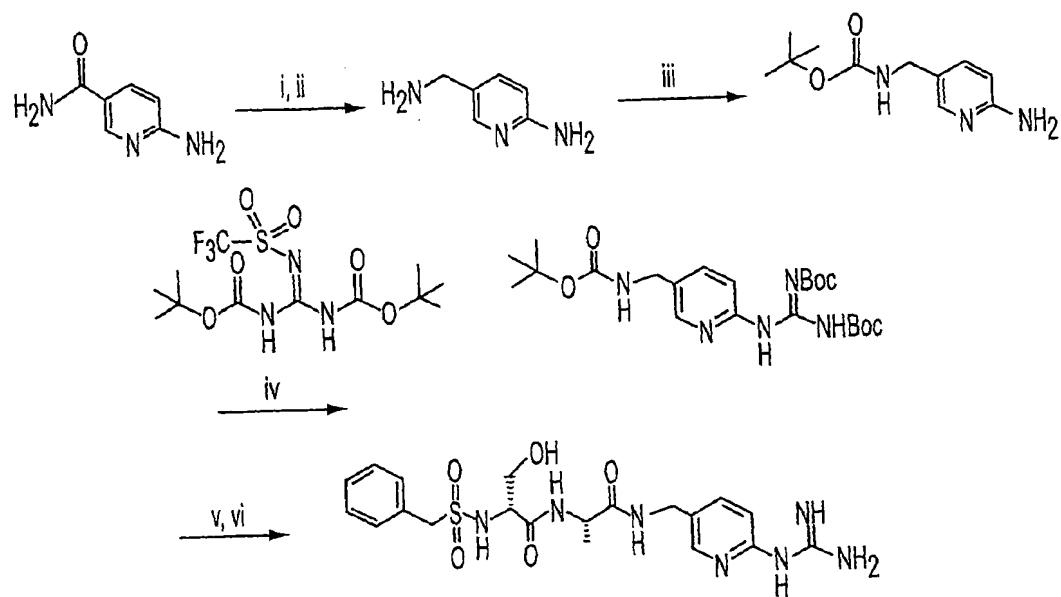


FIG. 17

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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WO 02/014349 A3

(51) International Patent Classification⁷: **C07K 5/062**, (74) Agent: **BIGGS, Suzanne, L.**; Lyon & Lyon LLP, 633 W. 5th Street, Los Angeles, CA 90071 (US).

(21) International Application Number: **PCT/US01/25337**

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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(25) Filing Language: English

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(30) Priority Data:

09/637,483	11 August 2000 (11.08.2000)	US
09/733,645	7 December 2000 (07.12.2000)	US
00126874.7	7 December 2000 (07.12.2000)	EP

(71) Applicant (*for all designated States except US*): **CORVAS INTERNATIONAL, INC.** [US/US]; 3030 Science Park Road, San Diego, CA 92121 (US).

Declaration under Rule 4.17:
— of inventorship (Rule 4.17(iv)) for US only

(72) Inventors; and

Published:

— with international search report

(75) Inventors/Applicants (*for US only*): **LEVY, Odile, Esther** [US/US]; 5304 Ruetter De Mer, San Diego, CA 92130 (US). **MADISON, Edwin, L.** [US/US]; 11005 Cedarcrest Way, San Diego, CA 92121 (US). **SEMPLE, Joseph, Edward** [US/US]; 9711 Caminito Pudregak, San Diego, CA 92131 (US). **TAMIZ, Amir, P.** [US/US]; 3920 Ingraham Street, San Diego, CA 92109 (US). **WEINHOUSE, Michael, I.** [US/US]; 3215 Purex Road, Escondido, CA 92029 (US).

(88) Date of publication of the international search report:
3 October 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/014349 A3

(54) Title: NON-COVALENT INHIBITORS OF UROKINASE AND BLOOD VESSEL FORMATION

(57) Abstract: Novel compounds having activity as non-covalent inhibitors of urokinase and having activity in reducing or inhibiting blood vessel formation are provided. These compounds have P1 a group having an amidino or guanidino moiety or derivative thereof. These compounds are useful in vitro for monitoring plasminogen activator levels and in vivo in treatment of conditions which are ameliorated by inhibition of or decreased activity of urokinase and in treating pathologic conditions wherein blood vessel formation is related to a pathologic condition.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/25337

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7 C07K5/062 C07K5/06 A61K38/05		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 7 C07K A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
CHEM ABS Data, WPI Data, PAJ, MEDLINE, BIOSIS, EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 01 96286 A (KUENZEL SEBASTIAN ; SCHWEINITZ ANDREA (DE); STEINMETZER TORSTEN (DE) 20 December 2001 (2001-12-20)</p> <p>the whole document</p> <p>---</p> <p>-/-</p>	<p>1-10, 13-19, 21, 24-29, 33, 35-41, 43,44, 46-48, 51-54, 58-61, 63,64, 66,68, 69, 71-74, 76,77, 79,81, 82,84-87</p>
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		<input checked="" type="checkbox"/> Patent family members are listed in annex.
<p>* Special categories of cited documents :</p> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> <p>*T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*&* document member of the same patent family</p>		
Date of the actual completion of the International search	Date of mailing of the International search report	
9 July 2002	18/07/2002	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Groenendijk, M	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/25337

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 96366 A (KUENZEL SEBASTIAN ; SCHWEINITZ ANDREA (DE); STEINMETZER TORSTEN (DE) 20 December 2001 (2001-12-20) the whole document	1-10, 13-19, 21, 24-29, 33, 35-41, 43,44, 46-48, 51-54, 58-61, 63,64, 66,68,69
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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 05245 A (CORVAS INT INC ;TAMURA SUSAN Y (US); BRUNCK TERENCE K (US)) 3 February 2000 (2000-02-03) the whole document ---	1-86
A	TAMURA @? S Y ET AL: "Synthesis and biological activity of peptidyl aldehyde urokinase inhibitors" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 10, no. 9, May 2000 (2000-05), pages 983-987, XP004199057 ISSN: 0960-894X the whole document ---	1-86

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 71-87 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: 1-46, 51-67, 71-80(all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple Inventions in this International application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the Invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-46, 51-67, 71-80(all partially)

In view of the large number and also the wording of the claims presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search over the whole scope of the claims is impossible. Particularly the present claims relate to an extremely large number of possible compounds, defined by a general structure lacking any constant domain and being itself defined by cascading meanings of every variable. moreover there appears to be an inconsistency between said definitions and the experimental data (e.g., see the definition of R2, e.g., being -(CH₂)₂₀H). Consequently, the search has been carried out (and hence is only complete) for those parts of the application which do appear to be clear (and/or concise), namely the examples and the compounds defined in the figures 1-24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

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